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Dr. Thayer*

In devoting the next few moments to the memory of William Sydney Thayer the Interurban Clinical Club performs a sad but proud rite. We who are continuing to meet together as he and the other founders of the Club intended we should, what have we to say that can add to the lustre of his life? No need for that. Ah, but what need there is for us to sit again with him and feel his strength, to gain from him a bit of that impervious courage which carried him steadily on through life's sad trials and triumphs so often burdened with obligations! What need there is for us to refresh our idealism from his endless supply, to revere with him the honor and nobility of the profession of medicine!

Much has been said about the great men of medicine. Talents that constitute the basic elements of the great physician are recognized and even expected by a well educated if somewhat credulous reading public. To us who look out over the same footlights, who help to set the stage and give the cue, who even play the same rôles before smaller and less important audiences, these attributes of greatness are seen with more discerning eyes. There is no surprise for us in the spectacle of a well read and learned physician whose experience with human nature under all conditions makes him tolerant as well as wise. Disregard of worldly gain, of personal health, of the privilege of privacy, selfsacrifice in short, is the rule rather than the exception. Dr. Thayer, however, possessed such qualities in proportions that are rare even among our medical saints. Physician he was always, gentleman always, poet always, soldier he was when need arose. Accounts of his accomplishments are written in the medical history of America. His friends need no list of his achievements nor of the honors he accepted so gracefully. But only those who shared his love could know the depth and understanding of his friendship. When illness laid its heavy hand on the household where I lived and hearts

* A minute presented at the 53rd biannual meeting of the Interurban Clinical Club, December 8, 1933, in Baltimore, Maryland, by Henry M. Thomas, Jr., M.D.

were sick, he came and understood and stayed long hours to share the sorrow, to give wise advice and leave strength with which to go on. His friendship was genuinely unselfish. Indeed, as one thinks of all his acts one realizes that the motives were invariably unselfish. His was a sense of values in which self occurred only as a part of the whole picture. Not for him to give—rather to share in partnership. The simplicity of his greatness was its hall-mark despite the subtlety of his soul.

Is it extreme to think of Dr. Thayer physician as really Dr. Thayer poetic artist whose medium was the practice of medicine? He wrote charming and inspired prose but his attention to poetry actually amounted to an avocation. He took his Russian poets with him in his general's portfolio through the World War. The fact that he read, translated and wrote excellent poetry is an interesting side-light on his character, but its particular significance, it seems to me, lies in the explanation it affords us of his attitude toward things medical. The practice of medicine became, under his touch, a thing of beauty. Then, too, into his doctoring he brought an almost exaggerated courtliness which seemed at times as though it must be affected. How could he be so tolerant toward stupidity in other doctors? How could he be so unfailingly generous to his colleagues and assistants in assigning credit for professional deeds? How could he be so punctiliously polite to everyone? And then his obvious attention to the formal dictates of dress! Could anyone unaffectedly achieve such completeness of apparel in its every phase, be it morning coat, grey cutaway, checked sack suit with red tie, or evening dress, always embellished by a fresh carnation, with cape and cane and silk hat always from a Parisian shop? And yet Dr. Thayer was no mere polished gentleman, charming and poetic in manner. He was beyond this a sensitive, idealistic and courageous perfectionist.

There are frequent examples of his courage, a form of courage which led him to speak his mind whatever the occasion might be; to stand clearly behind his convictions without regard for the wishes of others or what they might think of him. And so disinterested were his motives, so selfless his actions, that feelings were not hurt. Even in the heat of argument one could not be offended by Dr. Thayer. It was as though he were propounding some religious doctrine that

transcended all personal feelings. His courage did not always make life easier for him. Just after he had undertaken a mission to Russia, then in the throes of revolution, his wife was overtaken by what both he and she knew was to be her last illness. Together they faced this great crisis and then off he went fully conscious that his return would be a lonely one. So it was, but as an act of God Dr. Thayer accepted it bravely. And from his sorrow came the lovely poem he called "The Light That Was," and these lines:

> "But ah, how short the day—my light has passed,
> Has vanished as the sun that sets: and now
> Again in darkness and alone, I grope
> Along the sombre way that winds before."

To such a person the art of medicine must perforce have held infinite charm. Clinician he was in every sense, great clinician and teacher of clinical medicine by precept and example. He had the ability beyond most other physicians of eliciting all the physical signs of illness and the clinical sense built of long years of attention to interpret correctly these signs. He relied on his own powers of observation and he remained sceptical of any laboratory test which might disagree with his findings.

Medical students learned from him the need of painstaking attention to detail and of precise observations. As a fundamental principle he insisted on careful and accurate use of words. "What, Mr. Jones," he would ask of a student in a clinical demonstration, "do you mean by the term hyperresonant which you have just used?" And Mr. Jones, well coached by an assistant who knew Dr. Thayer's distrust of words which might not be understood by the user, would answer "A percussion note that is longer, louder and lower pitched than normal." He spoke of white blood cells as colorless corpuscles and in many other places he broke away from the ordinary medical terminology in an effort to stimulate clear thinking. He insisted on the use of simple measures of examination many of which had become neglected through interest in newer methods. One of these was the distention of the stomach with effervescing solutions in order to outline its borders and locate a tumor; another was the examination of a film of fresh blood under the microscope. And like most great clinicians he taught by example the importance of obtaining accurate description of the

symptoms from the patient, overcoming the patient's shyness or antagonism and helping the duller ones to say what they mean rather than what the doctor supposes they should mean. He was proud, one always felt, to hand on what he had received of knowledge or method from his former Chief, Osler, and his former Chief must have been proud in his turn of his worthy successor.

Dr. Thayer might have been called absent-minded; indeed his abstraction was so great that he would pass even his most intimate friend in the corridor of the hospital without a glance of recognition and on some days he lost his hat and overshoes four or five times. Fortunately, however, his goings and comings were watched over by careful and intelligent secretaries, who were constantly in league with his changing but always devoted assistants, and he never missed an important engagement. Important engagements came thick and fast, clinics and consultations, of course, but also meetings of the Board of Overseers of Harvard University, addresses in Paris given in French, addresses in London at the Bright Centenary and on other occasions, presidential addresses at the Association of American Physicians or the American Medical Association, and so on. His only recourse to freedom was to go "out of town" into his study, as in no other way could be escape demands on his time from patients or more often from other physicians. There was some doubt about these trips "out of town" if they came in ducking season and his assistants recognized as pathogomonic of an incipient ducking trip an increasingly severe laryngitis. There were smiles over the fact that the ducking blind usually effected a cure, especially when he shot more accurately than his fellow hunters. But who can smile for long at a man whose regard for things of the world is so slight that he can stop his important consultation practice time after time for trips abroad or to a ranch out West for two, three or four months, only to return to find himself actually pressed for money? He cared nothing for money; he merely lived as he knew how and money was a necessary evil. His estate at the time of his death, when thought of in the light of his distinction, was almost nothing. This was due partly to his lack of business sense or rather his disregard for it, and partly to his generosity which led him to provide for the education of many of his less fortunate relations. At various times he was forced to reduce his

budget and on several occasions even to give up his motor car. No one saw him bother over these matters, however, and he never seemed discouraged. No one ever thought of Dr. Thayer with pity and no one ever lightly imposed on his courtesy. The air of distinction which enveloped him was permeated with graciousness and charm. Who can forget the soft music of his voice when on staff rounds with finger tips together and head thrown back in rapt gaze he told of cases seen with Sir William Osler or of how he heard the passage of an embolus through the heart while a House Officer at the Massachusetts General Hospital?

We shall never know another Dr. Thayer. No, but we may count ourselves fortunate who felt the high influence of his noble spirit—that influence which perpetuates itself in an ever widening circle of students of the art of medicine. In one of his poems he wrote:

> "Now thou art gone and all is dark and cold;
> And yet not all, for as the mountain top
> Glows roseate when the sun has hid his face,
> So has thy love yet left an afterglow
> That bathes the darkening autumn of my days;
> So shall the memory of what thou wert
> Yet gild the summits of my rugged way,
> Yet guide me on the path that I must tread."

Now he too is gone but ever will the summits along the way be lighted by the glorious afterglow left as a legacy to medicine by Dr. William Sydney Thayer.

STUDIES ON THE ADRENAL

IV. The Oral Administration of the Adrenal Cortical Hormone and the Use of Fresh Glands Therapeutically

ARTHUR GROLLMAN[1] AND W. M. FIROR

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Submitted for publication November 1, 1933

The promising use of the hormone of the adrenal cortex in therapeutics renders it important to determine the best route for the administration of this material, and especially to arrive at a decision as to the possibility of giving it successfully by mouth. Furthermore, the high cost of the purified hormone and the comparative impotence of available commercial preparations make it very desirable to provide a simple but potent glandular product which would be readily available for oral use.

The oral administration of dried preparations of adrenal cortex has been used by a number of previous workers on the assumption that they were thereby administering sufficient hormone to elicit a therapeutic effect. We have, however, been unable to demonstrate the presence of appreciable amounts of hormone in extracts prepared from such materials.

The earliest evidence in favor of the efficacy of oral treatment as a replacement therapy for adrenal insufficiency was that of Rogoff and Stewart (8) and of Rogoff (9) who reported notable effects in patients with Addison's disease treated with a crude extract prepared by their method.

Britton, Flippin and Silvette (2) also attempted to determine the effectiveness of the cortical hormone when given by mouth. Although these authors conclude that the hormone is effective when so administered, their data are not conclusive. The four cats reported

[1] Aided by a grant from the Committee on Scientific Research of the American Medical Association.

on by these authors, survived for only 6, 18, 4, and 17 days, respectively. In only two cases, therefore, was survival prolonged beyond the average reported by Britton ($7\frac{1}{2}$ days) for untreated animals (1). Moreover, the excessive amounts of extract administered by Britton, Flippin and Silvette are inconsistent with their claims that only 3 to 5 times the intraperitoneal dosage is required for oral use, for the amounts used are many times those stated by Swingle and Pfiffner (10) to be necessary for parenteral injection. We have also been unable to confirm the claim of Britton and his co-workers as to their ability to resuscitate prostrated adrenalectomized cats by oral administration of extract. In our own experience only intraperitoneal or intravenous injections have made possible the resuscitation of cats or dogs in true adrenal insufficiency.

In order to determine the activity of the cortical hormone when administered orally as compared with the intraperitoneal or the intravenous routes, we studied the effects of a given extract prepared by our method (6). In our assay we utilized the white rat as described in previous papers (3, 6). This method of assay has proved to be convenient and accurate in our hands, for it makes possible the assay of relatively small amounts of material in a short period of time. Larger mammals such as the cat or dog, although useful for demonstrating the efficacy of a given hormonal preparation, are not suitable for assay work. Such animals obtained at random are relatively variable in their response to treatment as compared with a 30 to 40-gram rat, which we utilize, and the great amount of material necessary for large animals makes impossible the use of a sufficient number of individuals to exclude the normal biological variation.

Typical results of our experiments on the relative efficacy of oral administration and intraperitoneal injection are reproduced in table 1. Several groups of six rats each were utilized for each assay, but for the sake of brevity results on only three animals are reproduced in the table. All the animals were reared in the laboratory and maintained on a constant adequate diet. On day 1, bilateral adrenalectomy was performed as previously described (3) under amytal anesthesia, which markedly prolongs life as compared with ether anesthesia. In the table are given the daily weights. The first two groups were treated for 7 days with the same extract prepared as previously described (6).

The first group received 0.5 cc. orally, the second 0.1 cc. intraperitoneally, daily, in a single dose. For oral administration a small rubber uretheral cannula attached to a needle and syringe was passed into the stomach. With practice the passage of the cannula through the cardiac orifice of the stomach is easily felt.

As seen in the table, when the dosage was in the ratio of 1 to 5 equally good results were obtained in the case of oral and intraperitoneal administration. If less than five times the minimal intraperitoneal dose was given orally, retarded growth and a shortened period

TABLE 1

The efficacy of the oral route of administration of an extract of the adrenal cortex in maintaining growth in adrenalectomized rats

Days after adrenalectomy	1	2	3	4	5	6	7	8	9	10	11	12	13
Therapy utilized	Body weights in grams												
0.5 cc. orally daily for first seven days	36	37	39	41	42	43	45	46	46	45	43	41	Dead
	40	43	46	49	50	52	53	55	54	54	53	50	Dead
	33	35	36	38	40	42	43	44	47	46	45	Dead	
0.1 cc. intraperitoneally daily for first seven days	40	43	45	46	47	48	50	50	48	Dead			
	36	38	40	43	46	51	54	57	60	63	57	55	Dead
	30	32	35	38	40	42	44	45	46	48	50	50	Dead
0.5 cc. of 0.8 per cent NaCl intraperitoneally daily (Controls)	38	37	38	38	Dead								
	33	31	32	Dead									
	38	36	38	39	Dead								

of survival were noted. This ratio therefore represents the relative amounts necessary for oral administration as compared with injection. Although it is possible that differences in the digestive systems of various animals render this ratio inapplicable to man, it represents probably an approximation sufficiently accurate for practical purposes.

Comparative experiments on the relative efficacy of intraperitoneal and intravenous injections showed no apparent differences. This is not unexpected when one considers the relatively rapid rate of absorption of substances from the peritoneum.

ORAL FEEDING OF CORTICAL TISSUE

Having proved that the adrenal cortical hormone was absorbed from the gastro-intestinal tract, we continued our experiments in an effort to find a more economical and easily prepared form in which the hormone might be administered by mouth. Obviously the refinement of the extract as prepared for parenteral injection might be dispensed with and a cruder product be prepared suitable for oral administration. Our first efforts were confined to shortening the process used in making extract. Unfortunately, the whole adrenal gland is exceedingly toxic when ingested, as a result of the breakdown of epinephrine into highly toxic phenolic derivatives. Our crude extracts thus proved toxic unless great care was taken to dissect the cortex free of all medullary tissue soon after the slaughter of the cattle. Observing this precaution, the preparation of crude extracts involved almost as much trouble as the preparation of the pure extract (6).

Our next attempts were directed toward the use of glandular tissue without any attempts at extraction of the hormone. As previously shown by McKinley and Fischer (7) adrenal glands are very toxic when administered by mouth. However, by rapid removal of all medullary tissue immediately after slaughter of the animal, diffusion of appreciable amounts of epinephrine into the cortex is prevented and in this way a non-toxic material is obtained.

Fresh cortex thus obtained plus water *ad libitum* was given to adrenalectomized rats as their sole diet. In comparison with animals maintained on their normal diet there was no effect on survival. To avoid the criticism that the animals failed to recuperate from the operation sufficiently to recover their appetite for the glandular tissue, a series were treated with extract for several days (during which they grew normally) and then were placed on the glandular diet without evidence of any therapeutic effect. We furthermore confirmed the above results on rats by administering fresh glandular material to dogs. In no case did we find evidence of any therapeutic effect, the animals passing into insufficiency and dying in several days, as did untreated dogs.

The explanation of the failure of glandular feeding just described to have any therapeutic effect is attributable, as we have demon-

strated, to the rapid destruction of the hormone by enzymatic oxidative processes occurring spontaneously in glandular tissue. Contrary to the statement of Swingle and Pfiffner (10) we have found, by preparing extracts from glands at various times after removal from the body, that the hormone is rapidly destroyed. It was for this reason that we introduced the freezing procedure into our method (6) for preparing the extract.

In order to avoid this spontaneous destruction of the hormone, we therefore destroyed the enzymes responsible for this action. Imme-

TABLE 2

The effect of feeding various forms of cortical adrenal tissue on the survival of adrenalectomized white rats

Days after adrenalectomy	1	2	3	4	5	6	7	8
Therapy utilized	Body weights in grams							
"Enzyme-free" cortical tissue *ad libitum*	40	38	36	34	36	36	Dead	
	35	33	30	27	Dead			
	35	31	30	Dead				
0.1 cc. extract intraperitoneally daily for first 3 days: "enzyme-free" glands *ad libitum* thereafter	37	39	41	45	47	45	45	Dead
	38	39	40	37	38	38	Dead	
	33	35	38	39	38	Dead		
Controls on normal diet	32	31	32	32	Dead			
	35	35	37	36	34	Dead		
	35	36	37	Dead				

diately on removal from the cattle the glands were dissected and the medulla-free cortex was dropped into 95 per cent ethyl alcohol cooled by the addition of solid carbon dioxide (commercial "dry ice").[2] This procedure is similar to that used (6) in making our extracts, except for the substitution of non-toxic alcohol for acetone. The frozen glands were then transported to the laboratory, the alcohol decanted, and the glands ground and dropped into an equal quantity of boiling water. The concoction thus obtained, to which may be added salt or other flavoring materials, was administered to adrenalectomized rats and dogs.

[2] We, at first, utilized liquid air instead of alcohol and "dry ice" but its expense rendered its continued use prohibitive.

In table 2 are reproduced the results on rats. There is no evidence of any therapeutic effect derivable from such treatment. This failure we attributed to the fact that the rats refused to eat a sufficient quantity of the glands. The hormone present in at least 10 grams of cortex is necessary to maintain normal growth in a young rat; and since the animals ate only a small proportion of this amount, the negative results of table 2 are not unexpected.

We proceeded to feed the "enzyme-free" cortical tissue to dogs by forcing it into the stomach by means of a bulb and catheter, these animals, like the rats, having failed to consume, voluntarily, a quantity sufficient to elicit a demonstrable therapeutic effect. These attempts were successful, and we were able to prolong the life of several doubly adrenalectomized dogs by such treatment, as exemplified in the following protocol.

A female adult mongrel collie weighing 22.7 kilograms was bilaterally adrenalectomized at one sitting under ether anesthesia on April 20, 1933. On the day of operation and daily until May 12 (*i.e.* for 22 days) she was given by stomach tube 200 grams of "enzyme-free" cortical tissue. The dog remained in apparently good condition during this period, weighing 24.5 kilograms on May 12. On this day, however, signs of adrenal insufficiency appeared and she was given an intraperitoneal injection of hormone which brought about her recovery. From May 12 to May 20 she was given 250 grams of cortex daily, but on May 20 again manifested signs of adrenal insufficiency for which she was given an intraperitoneal injection. Following this, the oral dosage of cortex was increased to 300 grams daily. This dosage was maintained for a month (until June 20), and during this time the dog remained in perfect condition. In order to compare the oral dose of 300 grams of glands with the required intraperitoneal dose, the dog was then maintained for a third month by daily intraperitoneal injections of hormone equivalent to 60 grams of cortex. This ratio of glands required by mouth as compared with injection was thus, as in the experiments on rats, equivalent to 5 to 1.

Treatment of the above described dog was stopped on the ninety-first day after adrenalectomy. It died a fortnight later (on the one hundred and fourth day) with the typical signs of adrenal insufficiency—anorexia, loss of weight, muscular weakness, *etc.* The non-protein-nitrogen of the blood, taken immediately after death, was

136 milligrams per cent; the blood sugar was 18 milligrams per cent. Careful autopsy revealed no accessory cortical tissue. The peritoneal fat and connective tissue in the region of the adrenal sites was sectioned and microscopically examined. No trace of adrenal cortical tissue could be found.

The above protocol illustrates the possibility of prolonging life in a dog by the oral use of "enzyme-free" glands. Unfortunately, the great amount of material necessary renders the method much less satisfactory than intraperitoneal injection of purified hormone.

DISCUSSION

Our chemical studies on the hormone of the adrenal cortex, to be presented in a subsequent paper, explain how the hormone can be utilized by the organism when administered by mouth and the necessity of increasing the dose fivefold over the dose to be injected. The hormone is non-protein in nature; hence, unlike insulin, it would not be subject to proteolysis in the gastro-intestinal tract. The hormone is, moreover, of a relatively small molecular weight as evidenced by ultrafiltration studies carried out by the method previously described (4); hence, one would anticipate that the hormone would be readily absorbed from the small intestine and in part perhaps from the stomach. The hormone is relatively acid-stable as we have demonstrated by acidifying it to the approximate pH of the gastric contents for several hours. On the other hand, it is sensitive to alkali being inactivated in alkaline solution. This latter phenomenon may in part explain the necessity of administering a higher dose orally than by injection, for the alkaline reaction of the intestine suffices to destroy a great proportion of the hormone before it is absorbed. The possibility of non-absorption of the hormone by the gut due to the formation of an insoluble salt, must also be considered.

As to the therapeutic value of oral treatment, the high cost of purified extracts renders their oral use uneconomical at present. The use of "enzyme-free" glands prepared as described above may prove valuable, however, in patients manifesting a mild degree of adrenal insufficiency. The ease with which such preparations can be obtained as compared with the expense involved in preparing the extracts would recommend their use. The great amounts of material which

must be ingested obviously precludes their use in severe cases, in which, moreover, the occurrence of gastro-intestinal symptoms would render their oral administration impossible.

A relatively small quantity of the cortical hormone is derivable from the adrenals by our present methods of extraction. There can be no doubt that part of the hormone stored in the glands is destroyed before they can be removed from the body. The following observations indicate, moreover, that the quantity of hormone present in the gland is not large. Injection of fresh saline suspensions of cortex into rats and dogs sensitized to beef protein failed in every case to elicit any therapeutic effects because of the relatively large amounts of material necessary to supply an appreciable amount of the hormone. The adrenal cortical hormone is probably stored throughout the tissues. Thus we have been able to extract it in small quantities from the liver. Its occurrence in the urine (5) also probably results from its widespread distribution throughout the body. The fact that animals survive some days after adrenalectomy also speaks for the existence of this body store of hormone. The adrenals need not, therefore, contain a large store of the formed hormone but might satisfactorily perform their function by slowly liberating it to the blood from which it is absorbed by the tissues. There being no need for a sudden discharge of its product no large ready formed store of the hormone in the gland would be essential.

SUMMARY

The oral replacement dose of the adrenal cortical hormone is five times that required by parenteral injection. The hormone, being non-protein in nature and fairly stable in acid solution, is not affected by the stomach. It is rapidly inactivated in an alkaline medium, which accounts probably for its destruction in the intestine and the necessity of utilizing the larger oral dosage.

Fresh or dessicated adrenal tissue is highly toxic unless the medulla is completely removed immediately after removal of the glands from the body. Such glands are ineffective therapeutically because of the rapid destruction of the hormone by oxidative enzymatic processes. Cortical tissue rapidly heated to 100°C. preserves its hormonal content to a sufficient degree to offer a source of the hormone, as was demonstrated by feeding experiments in adrenalectomized dogs.

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SINO-AURICULAR BLOCK WITH VENTRICULAR ESCAPE

F. W. LIGHT, JR.

From the Electrocardiographic Laboratory of The Reading Hospital, Reading, Penna., Dr. W. S. Bertolet, Chief

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Sino-auricular block is a relatively rare condition. Compilations of reported cases have been made by Levine (1) in 1916 and Barlow (2) in 1927, totaling 54. Not included in these lists are 8 cases reported by Martini and Müller (3) in 1925. Since 1927 at least ten additional reports, with clearly corroborative electrocardiograms, have appeared (4, 5, 6, 7, 8, 9, 10, 11). The current textbooks show electrocardiograms illustrating sino-auricular block, some of which apparently are not taken from any of the papers just mentioned. Thus, no less than 75 cases of this condition have found their way into the literature.

The present case is peculiar in that there occurred escape of the ventricles, with short episodes of A–V nodal ventricular rhythm. Only a few electrocardiograms exhibiting this phenomenon have been published (1, 3, 12, 13). Isolated nodal extrasystoles, without the establishment of a real nodal rhythm, are more frequently noted in association with S–A block.

CASE REPORT

M. M., white, married, housewife, aged 45, was admitted to the Reading Hospital, on the service of Dr. I. H. Hartman, on July 21, 1931, complaining of shortness of breath, swelling of the ankles and palpitation of the heart.

The patient stated that she had had scarlet fever and rheumatism in childhood. Otherwise, her past history contained nothing of note. Three months before admission, she had some rheumatism, not severe enough to prevent her carrying out her usual activities. (Both attacks of rheumatism were characterized by swelling of the fingers, elbows and knees.) Only a week before coming to the hospital, she began to suffer from dyspnea, even on slight exertion. A few days later, edema of the feet and ankles appeared, this being most marked at the end of the day. These complaints became rapidly more pronounced.

On admission the temperature was 99°F., pulse 120, respirations 40 and blood pressure 166/108. Physical examination showed a very obese woman, having con-

siderable difficulty in breathing, even though sitting up in bed. There was slight cyanosis of the lips. The pupils were regular and equal, and reacted sluggishly to light. The upper teeth were missing, the lower in poor condition; the tonsils were large, but not grossly diseased. There was no enlargement of the thyroid. The chest expansion was poor, but equal on the two sides. Many moist râles were heard at the right base. The heart, on percussion, was found to be enlarged slightly to the left; the heart sounds were very distant, and no murmurs were audible. No organs or abnormal masses were felt through the very obese abdominal wall. Marked edema of both legs was noted, as well as varicosities of the superficial veins and some scars of old healed varicose ulcers. X-ray examination of the chest revealed cardiac enlargement of the aortic type and pulmonary congestion. The blood Wassermann was negative. The only laboratory findings of note were: a "heavy cloud" of albumin in the urine; blood urea N, 32 mg. per 100 cc.; blood NPN, 63; white blood count, 18,400.

Under treatment, the patient showed gradual improvement. The principal drug employed was digitalis. Until the arrhythmia which is the subject of this report was noted (a period of 18 days), she was given, altogether, the equivalent of 4.6 grams of the dried leaf. On a number of occasions, vomiting followed this medication. The pulse gradually dropped to about 85. After the arrhythmia appeared, the dose of digitalis leaf was reduced to 0.1 gram daily, which was well borne. It appears that the S-A block, to be described presently, was the result of overdigitalization, a frequent etiological factor in these cases.

The first electrocardiogram was taken the day after admission. The rate was 135, the P-R interval .16. QRS was slurred in all leads. T_I was sharply inverted, with slight upward convexity of the S-T segment in lead I; T_{II} was flat and T_{III} upright. A deep Q_{III} was present. On August 7, when the arrhythmia was first noted, typical digitalis T-waves were found in leads I and II (fig. 1). On August 18, essentially the same picture prevailed in the electrocardiogram. No later electrocardiograms were taken previous to discharge.

When discharged on August 24, 34 days after admission, the patient had no edema or dyspnea. She was seen again in the out-patient department on January 22, 1932. At that time she stated that she had been feeling well since leaving the hospital. She had experienced no dyspnea on moderate exertion, and, indeed, had walked two miles to the trolley line that day without discomfort. There had been no noticeable edema since discharge, but her already ample weight had increased 25 pounds. An electrocardiogram taken at that time showed no more slurring of QRS complex than before, a P-R interval of .20 sec., and good-sized upright T-waves in all leads. A pronounced Q_{III} was again noted. (The admission and follow-up electrocardiograms do not exhibit the minute initial upstroke of the QRS complex in lead III which is seen in fig. 1.)

DISCUSSION

All the electrocardiograms here reproduced were taken on the same day, during the period in which the irregular rhythm prevailed. In

fig. 1 are seen the three standard leads. Figs. 2 and 3 are from lead II, with double the standard galvanometer sensitivity. The auricular rhythm is easily followed, its irregularity consisting of occasional dropped beats. At no time is more than one beat missed, but in all

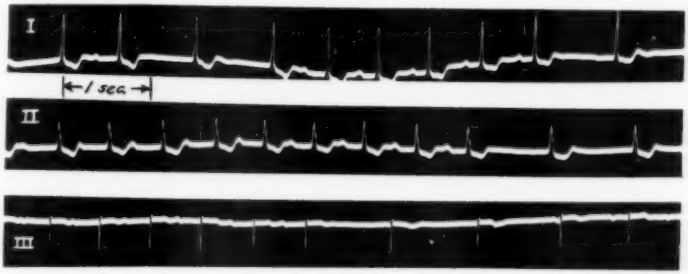

FIG. 1. THE THREE STANDARD LEADS. TAKEN SAME DAY AS FIGS. 2 AND 3

The electrocardiograms were taken with a Victor machine. The reproductions are from contact prints made from the original film.

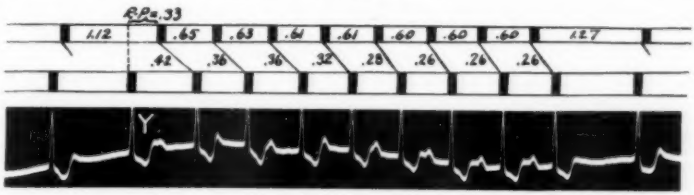

FIG. 2. Showing periods of S-A block, with escape of ventricles, and behavior of P-R intervals after short R-P. In the diagram the upper row of numbers indicates P-P intervals, the lower, P-R.

FIG. 3. ILLUSTRATING THE SHORT P-R'S FOLLOWING A LONG R-P

observed instances two or more auricular pauses succeed one another. In the portions of the record most closely approaching a normal picture, the P–P interval averages .61 seconds. The pauses vary from 1.11 to 1.29 seconds in length, thus approximating two normal P–P intervals. There is no regular relationship between the lengths of successive pauses; in some instances the first is longer than the second,

in others the second is the longer. In the series of beats preceding dropped auricular beats, the P–P intervals do not grow progressively longer; indeed, P–P tends to become slightly shorter before pauses. The first P–P after a pause, however, is definitely lengthened, being as high as .70 at times. These observations indicate that the S–A block in this case cannot be explained, in any simple fashion, upon the basis of a gradually lengthening S–A interval, assuming a constant sinus rate.

It will be noted that during the auricular pauses the ventricles begin to beat at a new rate. During regular auricular activity, the R–R interval averages about the same as the P–P, namely .61 seconds. When an auricular beat is dropped, a nodal ventricular rhythm appears, with an R–R varying from .91 to 1.00. All these escaped ventricular beats have a supraventricular form, with the exception of occasional extrasystoles.

The P–R interval varies within wide limits. Frequently it is high in the first beat after a normal mechanism is resumed, following a burst of nodal rhythm. The lengths of the P–R's of such beats can be correlated with the proximity of the preceding ventricular beat. This is shown in the following table, which includes all such beats present in a long record:

PRECEDING R-P	P-R OF INITIAL NORMAL BEAT
0.56	0.22
0.58	0.23
0.57	0.23
0.56	0 23
0.48	0 23
0.50*	0.24
0.47*	0.24
0.52*	0.25
0.53*	0.26
0.36*	0.27
0.38	0.29
0 34	0.42
0.33	0.42
0.29	0.44
0.25	0.46
0 24	0.46

* Preceding beat an extrasystole.

This relationship is readily explained through a consideration of the state of the A–V node when the impulse reaches it from the auricle. When a nodal or extrasystolic ventricular beat occurs after the A–V node has recovered from the last auricular impulse, it discharges the node, which then goes into its absolute, and subsequent relative, refractory period. When the auricular beat falls very shortly after such a nodal beat (very short R–P), the node is in the absolute refractory period and the impulse does not get through at all. This is illustrated by beat "X" in fig. 3. When R–P is a little longer, as in beat "Y" (fig. 2) the impulse from the auricle finds the node relatively refractory, and is conducted at a diminished rate, giving a long P–R interval. As R–P becomes longer, the auricular impulse finds the node in a more and more advanced stage of recovery. Thus, after the longest R–P's, the node is fully recovered, and a "normal" P–R results. In this patient, the "normal" P–R is unusually long, indicating that she is suffering from a delayed A–V conduction as well as from S–A block.

It is interesting to note that the R–P at "X" (fig. 3) is .24, the same as that preceding the longest P–R in the table, so that, with an R–P of that value, some beats get through the A–V node and some do not. When R–P is a little longer, as in the beats listed at the bottom of the table, the auricular impulse reaches the node at the very beginning of recovery. On the other hand, any R–P over about .50 permits complete recovery of the node, with a consequent "normal" P–R. The duration of the P-wave, as closely as can be determined, is .09 seconds; the interval between the end of P and the beginning of R, in the beats showing most rapid conduction, is .13. If the former value may be considered to represent the time of spread of the impulse through the auricle (referred to below as "M"), and the latter the time taken by it to traverse the A–V junctional tissue ("N"), the duration of the refractory period of the nodal tissue can be computed. It is necessary, also, to know where the impulse from the auricle is blocked and in what part of the node the nodal rhythm originates. It seems reasonable to suppose that the block occurs at the auricular end of the node, since it is produced by a retrograde impulse, and that the nodal rhythm originates at or near the ventricular end of the node. If it is further assumed that conduction through the node takes place at the

same speed in either direction, the absolute refractory period is seen to equal:

(Shortest R–P permitting passage of impulse through A–V node) +M −N.

In the present case, this gives as the duration of the absolute refractory period .20 seconds. The relative refractory period, calculated in a similar fashion, is found to be .46. Different assumptions as to site of blocking and of origin of nodal rhythm will, of course, give higher results.

A feature not so readily explained is the gradual diminution of P–R from beat to beat after the resumption of normal rhythm. This occurs quite regularly, and is illustrated in fig. 2. It seems necessary to postulate some depression of the A–V node resulting from the passage of an impulse before full recovery has taken place. The mere blockage of an impulse does not produce this effect.

The effect of drugs, vagal pressure or other measures was not investigated in this patient.

SUMMARY

There has been described a case of sino-auricular heart block, in which escape of the ventricles occurred. Certain peculiarities in A–V conduction are pointed out, which permit a calculation of the refractory period of the A–V node.

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THE RELATION OF ALLERGY TO IMMUNITY IN TUBERCULOSIS

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I. INTRODUCTION

It has been long believed that acquired immunity in bacterial infections in general, and in tuberculosis in particular, is dependent upon the hypersensitive state. Both the inhibition of spread of bacteria and their successful destruction have been assumed to result from the accelerated and exaggerated local inflammation which characterize the local reaction of the allergic body to the presence of the tubercle bacillus. Several years ago, Rich (1) (2) pointed out that there exists no valid evidence for the correctness of this assumption. In no infection, under any circumstance, has either the inhibition of spread or the destruction of the bacteria by the immune body ever been shown to depend upon the presence of allergy. The conclusion that allergy is necessary for the operation of immunity has been drawn chiefly from the observation that in tuberculosis in the guinea-pig, when immunity becomes demonstrable, allergy is also present. It is often stated, therefore, that "allergy parallels immunity." Rich and McCordock (3), however, have set forth numerous facts relating to tuberculosis in the human being and in experimental animals which do not agree with that statement, and other similar observations are continually being brought to light. As an example, we may mention the very recent paper of Pinner and Kasper (4) in which the results of a careful study of tuberculosis in the Negro "lead necessarily to the conclusion that infection tends to produce a high state of allergy in the Negro, but that he, unlike the white, does not readily acquire a state of increased resistance coincidently." This conclusion is entirely in harmony with the findings of the Tuberculosis Research Committee of the South African Institute for Medical Research (5), and will be

recognised as correct by everyone who has studied the relations of allergy and immunity in tuberculosis in the Negro. Surely, here allergy does not parallel immunity.

In view of these contradictions and discrepancies in the now widely held theory of the relation of allergy to immunity, it is of importance for the clarification of our knowledge regarding the nature of immunity to find out whether or not immunity actually is dependent upon the existence of allergy. But this issue is of importance also in another regard. It is well known that the hypersensitive state is responsible for destruction of tissue, for the mechanical spread of the infection, and also for many of the constitutional symptoms of infectious diseases. The solution of this problem may, therefore, have important implications in regard to the desirability of therapeutic desensitization in bacterial infections.

In a series of experiments directed toward the solution of this problem, Rich and his co-workers have recently shown in a variety of different infections that allergic inflammation is not necessary either for the inhibition of spread or for the successful destruction of bacteria in the immune body. It was demonstrated, first, that immunity in syphilis can be established without the concomitant development of allergy (6); second, that in pneumococcal infection immunity can be completely separated from allergic inflammation by the method of passive immunization (7); and, third, that when the hypersensitivity of animals immune and allergic to pneumococcal or pasteurella infection is abolished by desensitization, immunity remains intact in spite of the absence of allergy (8). In each of these types of experiment, both the inhibition of spread of the bacteria and their destruction occurred in the complete absence of allergic inflammation. It was then shown that the prevention of the immediate spread of bacteria from the site at which they lodge in the immune body is dependent primarily not upon inflammation but upon the action of the immune antibody (9) (10). These various experiments have made it clear that, in every instance in which immunity has been separated from allergy, immunity has remained intact in the complete absence of allergic inflammation, and, likewise, of allergic damage to tissue. However, since so much has been said and written about the necessity of allergy for immunity in tuberculosis, and especially since rational

treatment with tuberculin demands the knowledge whether or not the abolition of allergy by desensitization will impair the efficiency of immunity in tuberculosis, the experiments reported in this paper were carried out to test this matter directly. It may be said at once that the results of this investigation have demonstrated, just as in the case of the acute infections studied by Rich and Jennings, that immunity in tuberculosis is not impaired by the abolition of allergy.

This problem was undertaken with the full realization that it would be a difficult one, and it has indeed proved to have been so. The work has been in progress for a period of five years, for repeated series of experiments have been necessary in order to secure properly controlled results. Realizing the many possible sources of error in work of this sort, we have endeavored continually to perfect the methods of control, in order that we might obtain a series of observations which would answer definitely the question which we were investigating. We are quite aware of the fact that it is widely believed that tuberculous guinea-pigs cannot be made to tolerate large doses of potent tuberculin, and also that it is stated that even if desensitization be accomplished, hypersensitiveness will return on reinfection. The present experiments will show that when desensitization is accomplished by a very gradual increase in the dosage of tuberculin, reactions being avoided as far as possible, the animals can tolerate for weeks amazingly large daily doses of potent tuberculin; and that if these large doses are continued daily after reinfection with virulent bacilli, hypersensitiveness never appears. If desensitization is attempted by too rapid increase in dosage of tuberculin, the animals will invariably waste and die; and if reinfection is performed before desensitization is complete,—*i.e.* before the animals are able to tolerate very large doses of tuberculin—hypersensitiveness will indeed return. If hypersensitiveness returns after reinfection in the face of continued daily injections of tuberculin, it invariably means either that complete desensitization has not been accomplished, or that the doses of tuberculin are not sufficiently large to maintain desensitization in the face of reinfection.

We wish to take this occasion to express our very deep indebtedness to Dr. Arnold R. Rich without whose advice and encouragement it would have been impossible for us to have brought these experiments to a successful conclusion.

II. NATURE OF COMPARISON TO BE MADE

In attempting to put to a direct test the part which allergy may play in relation to resistance to tuberculous infection, the following general plan was adopted. A group of guinea-pigs were infected in the groin with 0.2 cc. of a milky suspension of avirulent tubercle bacilli of the Saranac strain known as R 1. It is well known that, except in very rare instances, animals infected with this strain of organisms do not develop progressive tuberculosis. Following such inoculation lesions are found at the site of inoculation and in the regional lymph nodes, and occasional solitary tubercules are to be found in the spleen and lungs, rarely in the liver. As has been established by numerous workers, and repeatedly confirmed by us, animals so inoculated become, after a lapse of a few weeks, relatively immune to re-infection with virulent organisms. The immunity is not absolute and with an adequate dosage of virulent tubercle bacilli, they suffer from a slowly progressive infection, the characteristics of which will be described below, but in any case they survive for much longer periods than non-immune animals injected with virulent bacilli. At about the same time at which immunity to re-infection is shown, evidence of allergy to tubercle bacilli and to their protein products becomes manifest.

When allergy to tuberculin had been well established in the animals immunized with R 1 bacilli, one half of the group was given daily doses of tuberculin O. T. injected subcutaneously. The injections were continued in increasing amounts until all evidence of the allergic state had disappeared. The dosage of tuberculin and the criteria for the suppression of allergy by which we were guided will be given in detail below. For the present, it may be stated that the degree of desensitization achieved in several series of animals was complete by all the criteria which we could apply. This tuberculin-treated group of animals will hereafter be spoken of as the "*immune desensitized*" group, and it was proposed to test the degree of the immunity of these animals in comparison with that of the "*immune allergic*" group of animals which had been inoculated at the same time with the same amount of the R 1 strain, but which had received no desensitizing treatments. The character of the test inoculation with virulent bacilli and the criteria by which the resistance was gauged will be discussed below. The daily injections of tuberculin administered to the *immune*

desensitized animals were continued during the whole course of the experiment following reinfection in order to prevent the recrudescence of allergy.

III. NATURE OF CONTROLS

(*a*) *Normal controls*

We have carried out four major series of experiments as outlined above. In all instances, a group of normal animals were inoculated with the virulent bacilli in the same manner and at the same time as the two groups already mentioned. These normal, non-immune animals were included in the experiment in order to make certain that the strain of organisms used was really virulent and in order to have a basis of comparison for the degree of immunity of the allergic and non-allergic immunes. This group will be called the "*Normal Control*" group.

(*b*) *Broth controls*

In addition, it was felt that the daily injections of large amounts of tuberculin might, either as a result of the mechanical handling of the animals, or as a result of non-specific effects of the injected material, affect their immunity. In various series of the experiments, therefore, all or part of the *immune allergic* group were given daily injections of some foreign solution in amounts equal to the daily injections of tuberculin in the *immune desensitized* group. One such group received injections of Petroff's glycerine broth concentrate prepared as follows. The broth was the standard culture medium used to prepare the tuberculin O. T. Without inoculating the broth with tubercle bacilli, it was concentrated to one tenth its original volume by evaporation in a vacuum still. It was then filtered through a Berkefeld N filter and stored in sterile bottles. Tuberculin O. T. is, of course, the filtered broth in which bacilli have been grown, similarly concentrated. Daily injections of this broth concentrate were given to one group of *immune allergic* animals in volumes equal to the corresponding dose of tuberculin O. T. given the *immune desensitized* animals. The animals receiving the injections of broth will be spoken of as the "*Immune Allergic Broth Controls*" or more briefly as the "*Broth Controls*."

Prior to reinfection with virulent bacilli, the general health of the

broth control group, as indicated by loss of weight, susceptibility to, and mortality from, intercurrent infection was worse than that of the corresponding tuberculin-treated animals. It is of great interest, however, that some of the animals of this *broth control* group suffered an appreciable loss of allergy to tuberculin after they had received injections of broth alone for several weeks. Owing to the disappearance of reactivity to test injections of tuberculin given intracutaneously, two of these animals were further tested by the intraperitoneal injection of 2000 milligrams of undiluted tuberculin O. T. Such an injection was, of course, regularly fatal to animals of the immune allergic untreated group, death occurring either in a few moments or after 6 to 8 hours. The *broth control* animals tested by this intraperitoneal injection showed little or no reaction and survived the test. This interesting accidental finding, exhibiting the possibility of non-specific desensitization of tuberculous guinea-pigs requires further investigation. Since, however, our prime interest in the present study was the comparison of the degree of immunity in desensitized and non-desensitized animals, the use of a *broth control* series was eliminated from subsequent experiments.

(*c*) *Glycerine controls*

In order to avoid the possibility of non-specific desensitization, and in order to control the effect of the glycerine content of the tuberculin used in desensitization, in a subsequent experiment one half of the immune allergic animals were given daily injections of glycerine in physiological salt solution as a substitute for the broth. Three different preparations of tuberculin O. T. used in desensitizing were analyzed for glycerine content and were found to contain 24 per cent, 30 per cent, and 40 per cent, respectively. A solution of 40 per cent glycerine in salt solution was, therefore, prepared and daily injections of this solution were given to a group of the *immune allergic* animals in amounts equal to that of tuberculin O. T. given to the animals in process of desensitization.

Prior to reinfection with virulent bacilli, the general health of the *glycerine control* group as measured by loss of weight, susceptibility to and mortality from intercurrent infection was worse than that of the untreated *immune allergic* animals but better than that of the

tuberculin-treated animals. In summary, then, during the period of desensitization the general health of the animals receiving tuberculin occupied a position midway between that of those which received injections of broth and those which received injections of glycerine.

(*d*) *Ultrafiltered tuberculin*

In one series of experiments we used purified tuberculoprotein carefully prepared according to the method described by Long and Seibert (11). The tuberculin so prepared was titrated against Koch's O. T. by intradermal inoculation in tuberculous guinea-pigs, and made up to a volume equally potent in producing cutaneous reactions as compared with Koch's O. T. This solution was used in one series of experiments in an attempt to desensitize a group of immune allergic animals. It was given in the same series of increasing daily doses that had previously been found effectively desensitizing in the case of Koch's O. T. It was found, however, that this ultrafiltered tuberculin, produced from growth on protein-free medium, was far less effective in desensitizing than was Koch's O. T. For this reason, the use of this solution was omitted from subsequent experiments except for skin tests in which respect, as will be noted below, it was found to be a very useful control.

IV. INJECTION OF VIRULENT BACILLI

(*a*) *Dosage*

When a satisfactory level of desensitization had been reached in the tuberculin-treated animals, an inoculation of virulent tubercle bacilli was given to this group and simultaneously to all the control groups of animals. In three of the series of experiments, the Saranac strain H 37 was used for this purpose, but, inasmuch as this strain lost some of its virulence in our laboratory, a freshly isolated virulent strain of human-type bacilli was used in the last series of experiments. The bacilli were collected from 3 weeks old Petroff slants and emulsified in physiological salt solution. Numerous bacterial counts on such suspensions filtered through various coarse and hard filter papers were made in an effort to standardize the dosage of bacilli. Two months, and again one month before the expected date of inoculating the whole group of animals with virulent bacilli, suspensions prepared in this

manner were injected into small test series of *immune allergic*, *immune desensitized*, and *normal* animals in order to discover what dosage would be adequate to strain but not to overwhelm the resistance of the *immune allergic* group. These injections were made into the groin, the skin, and the anterior chamber of the eye of each of these animals. As a result of these preliminary tests, the following dosage was chosen for the particular strain with which the final experiments were performed: The whole bacterial growth of five 3 weeks old Petroff egg-beef slants was emulsified in 20 cc. of physiological salt solution by grinding in a mortar for half an hour, the salt solution being added drop by drop. For the injection into the groin, 0.2 cc. of this suspension was used. For injection into the skin, 0.1 cc. of a 1 to 6 dilution of this suspension was used. For injection into the eye, 0.005 cc. of the filtrate of this suspension through a medium grade filter paper (approximately 1000 organisms, some in clumps) was used. The reasons for the choice of these sites of injection will be discussed in the next section.

The relatively enormous number of tubercle bacilli chosen for groin and intracutaneous injection, perhaps, requires some explanation. It is obvious that far, far smaller numbers of these virulent organisms would be capable of producing massive and rapidly fatal lesions in non-immune animals. If we had been content to demonstrate that the allergic and the desensitized animals both possessed a certain degree of immunity in comparison with the normals, a small dose of tubercle bacilli would have sufficed. Such a dosage could be chosen that almost all the *normal control* animals would have died within 6 weeks, while almost all of the *immune allergic* and of the *desensitized* animals would have been free from gross lesions in the same period. Such, indeed, was the result in one of our series of experiments. This result, however, showed only that the desensitized animals retained enough immunity to restrain a small number of bacilli; but it gave no information as to whether the degree of immunity of the desensitized animals was as great as that of the allergic ones. This information could be obtained only by a comparison of the reactions of the two groups to large doses of bacilli, and large doses were, therefore, used for this purpose.

(*b*) *Sites of inoculation*

The original inoculation of avirulent (R 1) bacilli was given subcutaneously into the left groin. The subsequent inoculation of virulent bacilli was given into the right groin, intradermally into the skin at the back of the neck, and into the anterior chamber of the eye. All the animals included in a single experiment were inoculated with the same suspension of bacilli on the same day. In making the inoculations, an animal from each group was inoculated in rotating order,—immune desensitized, immune allergic, glycerine or broth control, normal control, and so on—over and over again, until all the animals of each group had been inoculated. This was done in order to avoid the possibility that, as the result of sedimentation of the bacillus emulsion, one group of animals might receive a different dose of bacteria from another.

The heavy inoculation into the groin was given in order to facilitate the general spread of the infection. That given in the skin was for the combined purpose of testing the allergy of the animals (Koch Test, see below) and of providing a lesion resulting from a comparable dose of organisms in all groups of animals for clinical and histological study. It was felt desirable to add a further site of inoculation in which the progress of the local lesion could be observed more minutely and accurately from day to day by visual inspection. For this purpose, the anterior chamber of the eye was chosen. The injections into the eye were made with a microinjection syringe and a no. 28 gage needle. Under ether anaesthesia, the conjunctiva at the nasal margin of the cornea was grasped with a small mouse-tooth forceps. The eyeball was rotated nasally and the needle inserted first through the conjunctiva and then through the cornea at its temporal margin. Special care was taken not to touch either the iris or the lens with the point of the needle. Only 0.005 cc. of bacillus emulsion was injected. With this small quantity it was almost always possible to avoid leakage of aqueous following the withdrawal of the needle. With this technique, it was found that only 2 per cent of the inoculated eyes suffered from accidental pyogenic infection.

V. CRITERIA OF IMMUNITY

Inasmuch as complete immunity to virulent human type tubercle bacilli cannot be obtained in guinea-pigs and inasmuch as the infecting

dose used was very large, it was to be expected that practically all animals of the series would develop tuberculous lesions to some extent during the period of the experiment. Owing to the high mortality from intercurrent infection among the *immune desensitized* animals and also among the *glycerine* and *broth controls*, it was necessary to set a time limit of two to three months to the test period following the inoculation of virulent bacilli. Within this period, whenever an animal of one group died, an animal of each of the other groups was sacrificed for comparative pathological study. At the end of the three months' period, the remaining survivors were sacrificed. For this reason, as well as on account of the irregular incidence of intercurrent infection, mortality statistics on the groups are not of great value except in regard to the *normal controls*, all of which had died at a period when there were still a good number of survivors in the various immune groups. In order to gauge the relative severity of the infection in these groups, the following data were available for comparison: weights taken once each week, clinical character of the ocular lesions, gross and microscopic findings in the viscera, microscopic findings in the eyes and in the skin at the site of the intracutaneous inoculation.

The data in regard to these criteria on four successive experiments, each involving 60 to 120 guinea-pigs are too voluminous for detailed report. Furthermore, some of the experiments were incomplete, either because of unforseen termination by intercurrent epidemics, or because of injudicious selection of the dosage of virulent bacilli. For these reasons, the results of only the last experiment will be reported in detail. It must be stated, however, that the results of all three previous experiments, as far as they go, were consistently in accord with those of the final series.

VI. THE PROBLEM OF DESENSITIZATION

(*a*) *Technical difficulties*

Before reporting the experimental results in detail, some comment must be made on the problem of desensitization. We are by no means unmindful of the fact that numerous workers have in the past attempted to desensitize guinea-pigs to tuberculin without success, and we do not wish to be understood as suggesting that desensitization can be achieved in any casually undertaken experiment. Anyone

who has worked with guinea-pigs in experiments requiring prolonged observation is familiar with the devastating setbacks produced by epidemics of pneumonia and diarrhoea among these animals. The mere problem of keeping a group of guinea-pigs alive for six months during which they are subjected to daily injections presents no small difficulty. We found that if the desensitizing doses of tuberculin were increased too slowly, most of the animals died of causes other than tuberculosis before desensitization was completed. On the other hand, if the dose of tuberculin is increased too rapidly, the animals became dehydrated and malnourished. The size of the injecting needle with which the injections of tuberculin, glycerine, or broth are given has an important influence on the incidence of pyogenic skin infections. After numerous trials, we selected a no. 20 gage, one-inch, needle which was plunged $\frac{1}{2}$ to $\frac{3}{4}$ inch under the skin before the injection was given. Likewise, the volume of injected fluid of not more than 2.0 cc. was selected after repeated trials to minimize the frequency of skin sloughs. The sites of injection of tuberculin had to be chosen with care so as to conserve areas of skin for intracutaneous sensitivity tests. Isolation of animals infected with diseases other than tuberculosis had to be carefully carried out. Altogether, approximately 600 guinea-pigs were used in the four major experiments and the numerous control series required to determine these various technical points. In the first experiment, only 8 per cent of the tuberculin-treated animals survived the period of desensitization and were available for inoculation with virulent organisms, and the corresponding broth control group suffered a similar mortality. In the final experiment, 50 per cent of the animals of the tuberculin-treated animals reached the virulent inoculation and 30 per cent of these survivors lived for two months after their infection with virulent bacilli. During this final period, each of these animals received daily injections which amounted in the two months to more than 100 cc. of undiluted tuberculin O. T.

(*b*) *Dosage of tuberculin*

The tuberculin used in desensitization, except for the one trial with ultrafiltered tuberculoprotein mentioned above, was standard Koch's Old Tuberculin prepared according to the directions specified by

Willis (12). Some of the tuberculin was obtained from the U. S. Public Health Service in Washington. The remainder we manufactured ourselves. Each batch of tuberculin was titrated by intradermal test on a group of 3 to 5 immune allergic untreated animals and was found to produce a good positive reaction in doses of 3 milligrams. The daily dosage of tuberculin given in desensitizing in the last experiment is shown in table II. Rigid rules for the rate of increase in the dosage cannot be given. The general health, weight, and liveliness of the animals have to be taken into consideration; if the tuberculin is not forced too rapidly, the animals remain in good condition throughout the experiment, but if the dosage is increased too rapidly, the animals become emaciated and dehydrated, and frequently skin sloughs appear. Frequent graded intradermal tests were made and these served not only as a measure of the degree of desensitization that had been achieved, but also as a guide for the daily dose of tuberculin to be given.

(*c*) *Intracutaneous skin tests*

In performing the tests for cutaneous reactivity to tuberculin, Koch's Old Tuberculin was used, dilutions being made such that the required dose of tuberculin was contained in 0.1 cc. of fluid. As controls, similar dilutions of standard broth concentrate (see broth controls above) and of ultra-filtered, glycerine-free tuberculoprotein prepared from the products of tubercle bacilli grown on Long's protein-free medium (see ultrafiltered tuberculin above). In this way false positive reactions due to hypersensitivity to the proteins of the broth, or to the irritation of trauma or of glycerine, could be checked. Frequent comparisons were made between the tests on the tuberculin-treated animals and those on the allergic untreated group as well as on normal non-tuberculous animals. Records of all tests were made in terms of the diameter of the areas of redness, induration and necrosis, if such appeared. Tests recorded as negative were those in which the reactions by these criteria were no greater than those shown by normal, non-tuberculous animals. Tests recorded as positive were those in which the reaction to Koch's O. T. and to ultrafiltered tuberculoprotein exceeded that to broth.

TABLE I

DATE		GROUP I *Immune desensitized*	GROUP II *Glycerine controls*	GROUP III *Immune allergic*	GROUP IV *Normal controls*
1–22–32		Injected with avirulent tubercle bacilli R I	Injected with avirulent tubercle bacilli R I	Injected with avirulent tubercle bacilli R I	
2–18–32		Positive skin test 5 mgm. tuberculin	Positive skin test 5 mgm. tuberculin	Positive skin test 5 mgm. tuberculin	
2–19–32	Daily injections of tuberculin given	Daily injections of tuberculin begun	Daily injections of glycerin begun		
4– 4–32		Negative skin test 40 mgm. tuberculin	Positive skin test 5 mgm. tuberculin	Positive skin test 5 mgm. tuberculin	
4–18–32		Negative skin test 100 mgm. tuberculin	Positive skin test 5 mgm. tuberculin	Positive skin test 5 mgm. tuberculin	Negative skin test 100 mgm. tuberculin
4–20–32		Subcutaneous intradermal intraocular injections of virulent human tubercle bacilli	Subcutaneous intradermal intraocular injections of virulent human tubercle bacilli	Subcutaneous intradermal intraocular injections of virulent human tubercle bacilli	Subcutaneous intradermal intraocular injections of virulent human tubercle bacilli
4–22–32		Koch test (0.1 cc. heavy suspension of virulent tubercle bacilli injected intradermally) negative	Koch test (0.1 cc. heavy suspension of virulent tubercle bacilli injected intradermally) positive	Koch test (0.1 cc. heavy suspension of virulent tubercle bacilli injected intradermally) positive	Koch test (0.1 cc. heavy suspension of virulent tubercle bacilli injected intradermally) negative
4–29–32		Negative skin test 100 mgm. tuberculin	Positive skin test 5 mgm. tuberculin	Positive skin test 5 mgm. tuberculin	Positive skin test 5 mgm. tuberculin

	Daily injections of tuberculin given				
5– 5–32	Negative skin test 100 mgm. tuberculin	Positive skin test 5 mgm. tuberculin	Positive skin test 5 mgm. tuberculin	Positive skin test 5 mgm. tuberculin
5–16–32	Negative skin test 100 mgm. tuberculin	Positive skin test 5 mgm. tuberculin	Positive skin test 5 mgm. tuberculin	Positive skin test 5 mgm. tuberculin
6– 8–32	Koch test (as on 4–22–32) negative	Koch test (as on 4–22–32) positive	Koch test (as on 4–22–32) positive	Koch test (as on 4–22–32) positive
6–19–32	Intraperitoneal injection 2000 mgm. tuberculin No reaction	Intraperitoneal injection 2000 mgm. tuberculin Death in 6 hours	Intraperitoneal injection 2000 mgm. tuberculin Death in 6 hours	All animals dead with tuberculosis

VII. SUMMARY OF ONE EXPERIMENT

On January 22, 1932, 90 healthy male guinea-pigs weighing between 500 and 650 gm. were inoculated with the avirulent R1 strain (see table I). On February 17, 26 days later, the first intracutaneous tests were made with 5 mgm. of tuberculin. All these animals showed in 48 hours markedly positive reactions, areas of redness ranging from

TABLE II

*Table of daily injections of tuberculin**

DATE	AMOUNT	DATE	AMOUNT	DATE	AMOUNT
	mgm.		mgm.		mgm.
2–19–32	5	3– 8–32	120	3–25–32	800
2–20–32	5	3– 9–32	140	3–26–32	900
2–21–32	8	3–10–32	160	From 3–27,	1000
2–22–32	10	3–11–32	180	to 4–8–32	
2–23–32	12	3–12–32	200	4– 8–32	1100
2–24–32	15	3–13–32	225	4– 9–32	1200
2–25–32	18	3–14–32	250	4–10–32	1300
2–26–32	20	3–15–32	275	4–11–32	1400
2–27–32	25	3–16–32	300	4–12–32	1100
2–28–32	30	3–17–32	350	From 4–13,	1200
2–29–32	25	3–18–32	400	to 5–10–32	
3– 1–32	35	3–19–32	450	5–10–32	1300
3– 2–32	40	3–20–32	500	5–11–32	1400
3– 3–32	50	3–21–32	550	From 5–12,	1500
3– 4–32	60	3–22–32	600	to 5–28–32	
3– 5–32	70	3–23–32	650	5–28–32	1600
3– 6–32	85	3–24–32	700	5–29–32	1700
3– 7–32	100			5–30–32	1800
				5–31–32	1900
				From 6–1,	2000
				to 6–22–32	

* Koch's O.T. given subcutaneously.

1.0 to 3.0 cm. at the sites of inoculation of the O. T. and the ultrafiltered tuberculin, with only minute reactions at the sites of inoculation of the broth.

Following this test, daily injection of tuberculin was begun on 35 of these animals (see table II), corresponding injections of glycerine in salt solution on 24 others; the 20 remaining were not treated. On

March 16, at which time the tuberculin-treated animals were receiving 250 mgm. of Koch's O. T. daily, another series of skin tests was made. Twenty-seven of the *immune desensitized* group each received 20 mgm. of Koch's O. T. intradermally with corresponding amounts of control solutions. The average diameter of the reactions to tuberculin O. T. was 3.7 mm.; that to broth was 6.7 mm. Seventeen of these animals gave entirely negative tests to tuberculin, thirteen entirely negative to broth, eleven showed no reaction to either injection. Ten animals of the *glycerine control* group were tested at the same time with 5 mgm. of tuberculin and corresponding controls. The average diameter of the reactions to tuberculin was 12.5 mm.; all reactions to this small quantity of broth were negative. Eleven animals of the *immune allergic* untreated group were also tested with 5 mgm. of tuberculin and corresponding controls. The average diameter of the reactions to tuberculin was 14.5 mm.; all reactions to broth were negative.

In previous experiments, it had been found that when the subcutaneous daily dosage of tuberculin reached 50 mgm. the intracutaneous reaction was ordinarily negative to 3 mgm., faintly positive to 5 mgm., and strongly positive to 10 mgm. Similarly, when the daily subcutaneous dose had reached 100 mgm., negative reactions had been found to 5 mgm. intracutaneous tests; when the daily subcutaneous dose had reached 200 mgm., negative reactions were found to 15 mgm. intracutaneous tests, but slightly positive reactions to 20 mgm. When the daily subcutaneous dose reached 250 mgm., the cutaneous reaction to 20 mgm. was negative.

On March 25, the daily subcutaneous dose of tuberculin O. T. had reached 800 mgm. and skin tests were made with 40 mgm. of tuberculin and corresponding amounts of control solutions. After forty-eight hours, the average diameter of the reactions to Koch's O. T. was 11.7 mm., to ultrafiltered tuberculin 3 mm., and to broth 6.2 mm. It is evident that at this stage of the experiment the animals still reacted positively to 40 mgm. of O. T., but negatively to corresponding amounts of ultrafiltered tuberculin.

On March 30, the daily subcutaneous dose of tuberculin O. T. had reached 1000 mgm. and skin tests with 40 mgm. were repeated. After forty-eight hours, the average diameter of the reactions in 27 animals tested with O. T. was 3.3 mm.; with ultrafiltered tuberculin 3.1 mm.,

with broth 4.5 mm. The test was interpreted as negative to 40 mgm. Fifteen of the animals of the *glycerine control* group were tested at the same time with 5 mgm. of tuberculin. The average reaction to this dose of tuberculin measured 13.2 mm. in diameter, the average reaction to the corresponding amount of broth measured 4.5 mm. in diameter.

On April 6 and April 13, the tuberculin-treated animals were tested with 50 mgm. of tuberculin O. T. and corresponding control solutions, all showing negative reactions. Again, on April 18, when the daily subcutaneous dose had reached 1200 mgm., eight of the tuberculin-treated animals were tested with 50 mgm. intracutaneously and eight

TABLE III

Results of intradermal tuberculin tests immediately prior to virulent reinfection

GROUP	NUMBER OF ANIMALS	TEST DOSE OF TUBERCULIN	AVERAGE DIAMETER OF REACTIONS 48 HOURS AFTER INJECTION			COMMENT
			O.T.	Broth concentrate	Ultra-filtered tuberculin	
		mgm.	mm.	mm.	mm.	
Immune Desensitized	8	50	3.8	3.0	0.7	Negative
Immune Desensitized	8	100	4.9	4.9	3.5	Negative
Normal Controls	3	50	8.0	3.6	3.0	Negative
Normal Controls	3	100	7.6	6.6	4.6	Negative
Glycerine Controls	12	5	13.8	2.0	10.0	Positive
Immune Allergic	9	5	12.0	2.5	11.0	Positive

others with 100 mgm. Three normal non-tuberculous animals were tested with 50 mgm. and three others with 100 mgm., all with corresponding broth controls. In addition, twelve of the animals of the *glycerine control* group and nine of the *immune allergic* untreated group were tested with 5 mgm. tuberculin and corresponding control solutions. The average diameter of the reactions to these tests is shown in table III.

Since the *tuberculin-treated* animals were now receiving the extraordinarily large daily dose of 1.2 cc. of tuberculin O. T. subcutaneously and showed completely negative reactions to 0.1 cc. of undiluted tuberculin O. T. injected intradermally, while the *glycerine control* group and the untreated *immune allergic* group still showed strongly

positive reactions to 5 mgm. of O. T. and desensitization of the tuberculin-treated animals had obviously been accomplished, the time was considered ripe for the injection of virulent tubercle bacilli. On April 20, two months after tuberculin treatment had been begun, all of these animals together with 40 normal animals were, therefore, inoculated in the groin, intracutaneously on the back of the neck, and intraocularly, with emulsions of virulent tubercle bacilli as specified above. The results so far as they refer to the progress of the tuberculous infection will be given below. The reactions to the intracutaneous injection of living virulent bacilli, however, throw light on the allergic state of the animals at this period of the experiment and will, therefore, be reported at this point, together with the results of a

TABLE IV

Results of intradermal inoculation of living tubercle bacilli at the beginning of the virulent infection

GROUP	NUMBER OF ANIMALS	AVERAGE DIAMETER OF REACTION 48 HOURS AFTER INJECTION	COMMENT
		mm.	
Immune Desensitized	21	0.4	Negative
Immune Allergic	11	7.5	Positive
Glycerine Control	17	12.1	Positive
Normal Control	40	0.5	Negative

similar intracutaneous bacillus emulsion test performed on a small number of animals three days previously. The results of these tests are in complete accord with those of the intradermal tuberculin tests performed at the same period. The *immune allergic* untreated animals and the *glycerine control* animals showed markedly positive reactions at the sites of injection of living bacilli. The *immune desensitized* or tuberculin-treated animals showed no more reaction either to tuberculin or to living tubercle bacilli than did normal non-tuberculous controls. In the latter, however, the minute local lesions present at 48 hours became progressively larger, whereas in the former the lesions never became as large. (See table IV.) The histological findings in relation to these tests with living bacilli will be given below (figs. 1 and 3).

FIG. 1. Skin reaction 72 hours following intradermal inoculation of virulent tubercle bacilli: A. Immune Allergic; B. Desensitized; C. Normal Control.

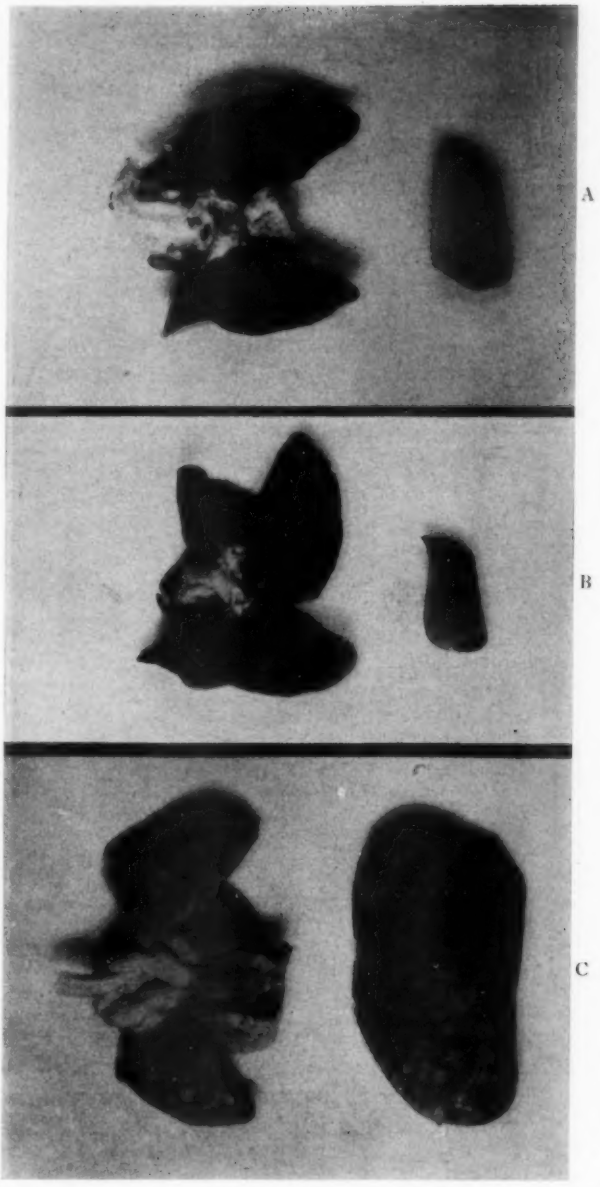
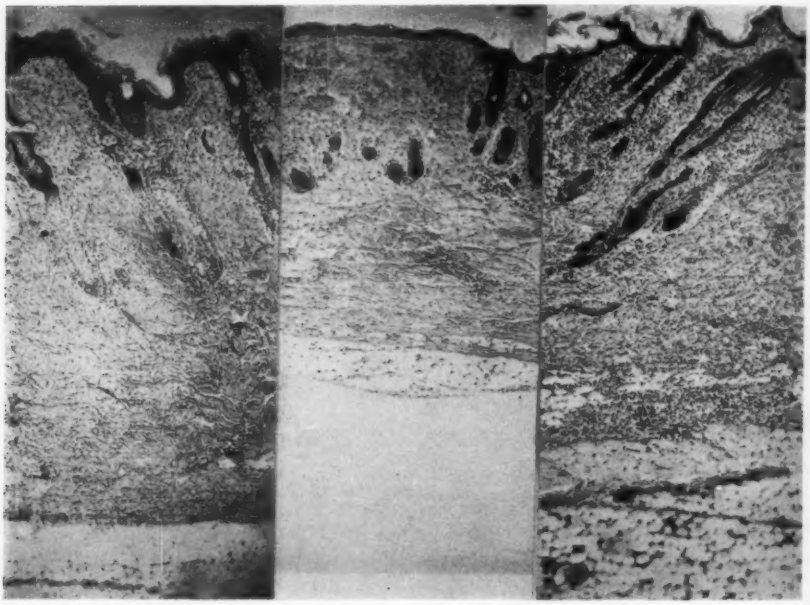


FIG. 2. Organs from a group of animals sacrificed 6 weeks after virulent infection: A. Immune Allergic; B. Desensitized; C. Normal Control.

On April 29, nine days after the inoculation of the virulent tubercle bacilli, the desensitized group were receiving a daily dose of 1200 mgm. of O. T. Intracutaneous tests were repeated. The results are shown in table V. It is to be remembered that the group labelled *normal controls* had never received an immunizing dose of R 1 bacilli, but had



A B C

FIG. 3. Skin reactions 48 hours after intradermal inoculation of virulent bacilli: A. Normal Control showing slight oedema and slight diffuse cellular infiltration extending down into the subcutis; B. Desensitized showing slight cellular infiltration in the cutis without appreciable oedema; C. Immune Allergic showing marked oedema and diffuse cellular infiltration extending down to the subcutaneous fat.

now been infected with virulent tubercle bacilli. The results of this test indicate that the desensitized animals remained desensitized in spite of having been re-infected with virulent organisms, the allergic animals remained allergic, and the *normal controls* already showed a high degree of allergy even at this early date, a result attributable to the large inoculating dose.

It would be tiresome to repeat in detail the results of similar skin tests repeated at intervals of every two to three weeks. The subcutaneous dose of O. T. given to the *immune desensitized* group was gradually raised to 2000 mgm. daily, as shown in table II. These animals continued to show negative reactions to the intracutaneous injection of 0.1 cc. of undiluted O. T. and of similar amounts of ultrafiltered tuberculoprotein. The allergic animals, both those that were untreated and those given glycerin, remained highly allergic. The *normal control* group, as long as survivors were available for skin tests, likewise continued to show a strongly positive reaction to 5 mgm. of O. T.

On June 8, nearly 4 months after tuberculin treatment had been begun, and seven weeks after their re-infection with virulent organisms, another intradermal test with an emulsion of living virulent bacilli

TABLE V

Results of intradermal tuberculin tests nine days after virulent reinfection

GROUP	NUMBER OF ANIMALS	TEST DOSE OF TUBERCULIN	AVERAGE DIAMETER OF REACTIONS 48 HOURS AFTER INJECTION			COMMENT
			O.T.	Broth concentrate	Ultrafiltered tuberculin	
		mm.	mgm.	mm.	mm.	
Immune Desensitized	13	100	6.0	5.9	5.8	Negative
Glycerine Controls	7	5	13.0	4.2	11.0	Positive
Immune Allergic	4	5	12.0	5.0	10.3	Positive
Normal Control	8	5	13.5	4.0	12.0	Positive

was performed on a small group of animals. The average reaction of the *immune desensitized* animals forty-eight hours later measured 4.4 mm., that of the *immune allergic* untreated animals measured 18.3 mm., that of the *glycerine controls* measured 16.0 mm., that of the *normal controls* (seven weeks after infection with virulent organisms) measured 25.0 mm., while a similar test on normal non-tuberculous animals measured 3.0 mm.

Finally, on June 14, eight weeks after the virulent inoculation, two *immune desensitized* animals, together with one *glycerine control* and one *immune allergic* were each given 2 cc. of undiluted tuberculin intraperitoneally. The two former survived and showed no signs of distress. The two latter became acutely ill immediately after the injection and died, respectively, eight and six hours later.

In summary, it may be said that the tuberculin-treated animals were actually desensitized at the time they were inoculated with virulent bacilli and that they remained desensitized during the following two months at the end of which time all survivors were sacrificed. Throughout this period they showed no more allergic reaction to the intracutaneous injection of undiluted O. T. or of a heavy emulsion of living virulent tubercle bacilli than do normal non-tuberculous animals, and at the end of this period they were able to tolerate without symptoms the intraperitoneal injection of doses of tuberculin far in excess of the amount which is rapidly fatal to non-desensitized tuberculous guinea-pigs. When it is further realized that these animals were receiving the extraordinarily large daily subcutaneous injection of 2000 mgm. of tuberculin O. T., again an amount far in excess of that which is fatal to an allergic animal, the degree of their desensitization becomes obvious.

VIII. RESULTS

(*a*) *Gross pathology*

As noted above, once the inoculation of virulent bacilli had been given, every effort was made to obtain rigidly comparable material from the various groups of animals for pathological study. Whenever an animal of one group died, one of each of the other three groups was sacrificed. In order to reduce this voluminous material to somewhat more manageable bulk, we have eliminated from the present discussion all animals that died or were sacrificed within the first 20 days after the virulent infection, for the amount of tuberculosis in the allergic and desensitized animals was always negligible up to this period. Furthermore, since the *normal control* group exhibited, as was to be expected, a high degree of uniformity in the massive character of their tuberculous lesions, they do not require very detailed comment. The greatest number of this group died in the fifth week following their inoculation. Of the forty inoculated, only two survived longer than six weeks, one dying on the 53rd, and one on the 59th day after infection. None surviving for 20 days or more were free from extensive visceral lesions. They showed great caseous lymph nodes in the right groin, greatly enlarged spleen flecked with innumerable small and

large tubercles, widely disseminated tubercles in the liver, large caseous peribronchial lymph nodes and scattered lesions of various sizes in the lungs. Characteristically, the lesions in the lungs were, on the whole, less widespread than those in the liver and spleen. There is no reason to specify further the familiar picture of the results of a massive tuberculous infection in non-immune guinea-pigs.

The immune animals of all three groups,—*immune allergic*, *immune desensitized* and *glycerine controls* taken together exhibited marked contrasts in comparison with the *normal controls*. Only two of these animals, one a *glycerine control* and one an *immune allergic*, showed a dissemination of lesions in any way comparable with that of the *normal controls*. In the great majority of these animals, the lymph nodes in the right groin were enlarged and caseous. Aside from the two individuals already noted, the great majority showed no lesions whatever in the liver and the remainder only an isolated small tubercle in this organ. The most prominent lesions found in this group of animals were usually in the spleen, but many animals showed no more than a single small hard tubercle in this organ such as is likewise to be found in animals inoculated with the R 1 strain without subsequent virulent inoculation.

Next to the spleen, the most prominent lesions were found in the lungs where the lesions ranged from isolated small solitary tubercles or even no visible lesions in some cases, to larger macroscopic caseous areas in a few instances. The bronchial lymph nodes on the whole showed lesions equal to or even in some instances surpassing those found regularly in the normal controls. This reversal of the relative severity of the lesions in the lungs and liver between the immune and non-immune animals merits some comment. Obviously, in order for the infection to go from the lymph nodes of the groin to the liver, the bacilli must pass through the capillaries of the lungs. The non-immune animal, however, lacks the ability to fix the infection locally in the lungs and the bacilli can pass on to be stopped by the reticulo-endothelial phagocytes of the liver and spleen. The ability of immune animals to accomplish the local fixation of bacteria has in the past been attributed to the allergic reaction. Rich, as already noted, has shown in pneumococcal infection that the mechanism of local fixation is independent of the allergic reaction. The fact that the completely

desensitized non-allergic animals in our experiments showed precisely the same sparing of the liver and dominance of the pulmonary lesions as did the allergic immunes may be offered as further evidence that allergic inflammation is not necessary to effect local fixation of bacteria. So far as the selection or sparing of particular organs in the dissemination of the visceral lesions may be used as evidence of the power of the immune body to effect local fixation, no differences could be discovered between the desensitized and the non-desensitized immunes.

Taken as a whole the group of immunized animals exhibited most extraordinary evidence of their immunity in the face of the massive infecting dose given. Of 31 of these animals living for 20 days or more after the virulent infection, 17 showed only such isolated lesions as were to be expected from the R 1 infection alone, while only two showed such extensive tuberculous lesions as to justify the belief that they died of tuberculosis.

Comparing now the immunized groups among themselves, we find no profound difference between the *immune allergic* untreated and the *glycerine control* groups, but on the whole the lesions were somewhat more widespread, though no more severe in character, in the latter than in the former. This is just what one would expect from the fact that the general health of the *glycerine control* animals was worse than that of the untreated *immune allergic* animals. Many of them suffered from pyogenic infections of the skin as a result of their daily injections, and they fell a prey to intercurrent epidemic diseases in larger numbers than did the members of the untreated group.

Compared with the *immune allergic* group, the *immune desensitized* animals showed a decided advantage. In none of the latter did we find the extensive caseous lesions which were the characteristic of the *normal controls* and of which isolated instances were found among the *immune allergic* and the *glycerine control* groups. None even showed severe and widely disseminated lesions. On the contrary, the great majority of these animals reacted little more than was to be expected from the R 1 infection alone. This is all the more remarkable when one remembers that the general health and incidence of intercurrent disease was worse in this group than even in the *glycerine control* group. A typical set of organs is shown in fig. 2.

A more specific analysis of the general severity of the infection in

these animals than the general impression given above is very difficult to present. The accompanying photographic illustrations of the gross organs gives only a feeble impression of their fresh appearance. We have attempted to grade the severity of the lesions by several different methods. In the first place, without knowing from which animals the organs came until the decision was made, the severity of the lesions in each organ was graded from 1 to 5, 1 being the least grossly visible evidence of tuberculosis, 5 being the most severe lesion in the whole group. Separate notes were made in each case in regard to lungs, bronchial lymph nodes, liver, and spleen. Subsequently, microscopic sections were made, carefully taken to include the most severe lesions in each organ, and these were reviewed and graded again without knowledge from which group they had come. A comparison of these two examinations led to the discovery of a few lesions not recognised in the gross specimens and to the correction of a few mistakes in which lesions not due to tuberculosis had been characterized on gross examination as possibly tuberculous. In all doubtful cases the decision as to whether or not a given lesion was tuberculous was fortified by the study of sections stained for bacilli by the Kinyoun method. These corrections entailed a slight shifting of grades for a limited number of organs, but did not in any way alter the general picture. The results of this grading are tabulated in table VI.

The material thus obtained can be classified in many ways, of which we present three. (1) The autopsies were grouped by the duration of life following the virulent infection, and each small group was arranged into sub-groups of minimal, moderate, and severe lesions, an extra group being added to take care of the massive lesions of the *normal controls* and of the two allergic animals who showed similar lesions. The results of this grouping are shown graphically in chart 1 and numerically in table VII.

(2) In the second place, the autopsies were grouped irrespective of the duration of the virulent infection into four similar groups of minimal, moderate, severe, and massive. This results in finding more "slight" cases among those of shortest duration and more "severe" ones among those of longest duration. The net result of these shifts happens to leave the numerical count as given in table VII unchanged.

(3) Since the severity of the lesions was recorded as from 0 to 5, it

TABLE VI

The severity of the visceral lesions in animals surviving over twenty days after virulent inoculation

NUMBER OF GUINEA PIG	GROUP	GROSS PATHOLOGY				
		Days after injection of virulent bacilli until death	Lungs	Tracheobronchial lymph nodes	Liver	Spleen
4– 6	Desensitized	65	+++	+++	Negative	++
12–16	Immune allergic	65	+++	+++	Negative	++
60–65	Glycerine control	65	+++	+++	Negative	+
37–41	Glycerine control	65	++++	++++	++++	++++
19–23	Immune allergic	65	++++	+++	+	++
21–25	Desensitized	60	Negative	+	Negative	Negative
46–50	Immune allergic	60	+	+	Negative	++
71–83	Desensitized	58	Negative	±	Negative	Negative
47–51	Immune allergic	58	+++	+++	Negative	+++
58–63	Glycerine control	58	++	+	Negative	++
14–15	Normal control	58	+++++	+++++	+++++	+++++
28–32	Glycerine control	56	+++	+++++	+++	++
18–22	Immune allergic	55	+	++	Negative	++
65–70	Desensitized	55	+++	++	++	++
64–69	Glycerine control	55	++++	+++	++++	+++++
67–72	Glycerine control	55	++	++	Negative	++
34–38	Desensitized	53	+	+	Negative	Negative
36–37	Normal control	53	++	+++++	++++	++++
56–61	Immune allergic	53	++	++++	Negative	++
61–66	Glycerine control	53	++	+++	Negative	+
78–90	Desensitized	39	+	+	Negative	+
31–35	Glycerine control	39	+	+++	Negative	+++
32–33	Normal control	39	++++	+++++	+++	+++
39–43	Glycerine control	39	+	+	Negative	+
5– 6	Normal control	37	+++	++++	++	+++
40–41	Normal control	37	++	++++	++	+++
58–59	Normal control	35	++++	++++	+++	+++
42–43	Normal control	35	++++	++++	+++++	++++
48–49	Normal control	35	+++	++++	++++	++++
64–65	Normal control	34	+	++++	+++	+++
51	Normal control	33	+++	+++	++	++++
44–45	Normal control	33	++	+++	+++++	+++
46–47	Normal control	32	+++	++++	+++	++++
75–87	Desensitized	31	Negative	Negative	Negative	+
45–49	Glycerine control	31	+	++	Negative	++

TABLE VI—*Concluded*

Number of guinea pig	Group	Gross pathology				
		Days after injection of virulent bacilli until death	Lungs	Tracheobronchial lymph nodes	Liver	Spleen
89–90	Normal control	31	++	+++++	++	++++
79–91	Desensitized	27	+	++	Negative	Negative
15–19	Glycerine control	27	+	+	Negative	Negative
20–24	Immune allergic	27	+	++	Negative	++
34–35	Normal control	27	+++	+++	+++	+++++
56–57	Normal control	27	+++	++++	+++	+++
74–75	Normal control	26	+++	+++	+++	+++++
29–33	Desensitized	24	Negative	++	Negative	Negative
16–20	Desensitized	23	+	+	Negative	Negative
7– 9	Glycerine control	23	++++	++++	++++	+++
55–60	Immune allergic	23	++	++	Negative	++
3– 4	Normal control	23	+++	++++	++	+++
33–37	Immune allergic	20	+	++	Negative	++
76–88	Desensitized	20	Negative	Negative	Negative	Negative
44–48	Immune allergic	20	++	++++	Negative	++
30–31	Normal control	20	+	+++	+	++
16–17	Normal control	20	+	++++	+	+

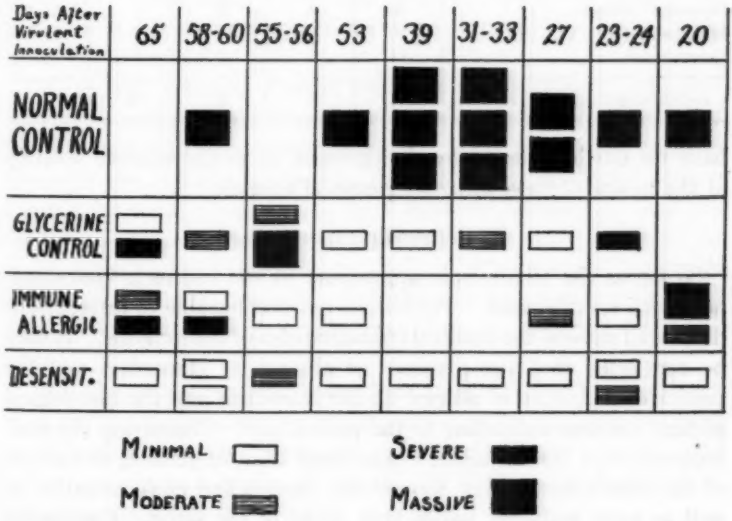


CHART 1. Chart showing the severity of the visceral lesions in animals surviving 20 days or more after inoculation with virulent tubercle bacilli.

was possible to add up the numbers indicating the severity of the affection of each organ and arrive at a number indicating, very roughly it must be admitted, the severity of the disease of each individual animal. Averaging this for each group, one obtains the following values for the severity of the lesions in the four groups of animals: *normal controls* 14, *glycerine controls* 8.4, *immune allergic* 6.8, *immune desensitized* 3.2. These numbers are not to be taken for more than they are worth. It is not meant by this that one group showed lesions twice as severe as another any more than the grades given to students on examinations are taken to mean that the man who is graded 100 knows precisely twice as much as the man who is graded 50. These figures merely indicate in as objective a manner as is possible the

TABLE VII

The severity of the visceral lesions in animals surviving over twenty days after virulent inoculation

Summary of data in table VI.

GROUP	NUMBER OF ANIMALS	MINIMAL	MODERATE	SEVERE	MASSIVE
Normal Controls	19	0	0	0	19
Glycerine Controls	10	4	3	2	1
Immune Allergic	10	4	3	2	1
Desensitized	11	9	2	0	0
All Immunes	31	17	8	4	2

basis for our judgments already expressed as to the relative severity of the lesions of these different groups of animals.

(*b*) *Microscopic examination*

So far as the microscopic appearance of the lesions is concerned, not much need be said. The lesions not attributable to intercurrent disease all showed the classical characteristics of tuberculosis. It may be concluded that the presence or absence of immunity, and the presence or absence of allergy, do not introduce into the histological picture features unfamiliar to the pathologist. Comparing the non-immunes with the immunes, it was found that, in general, the lesions of the former were softer, showed less fibrosis and more caseation as well as more stainable bacilli than those of the latter. Comparing

the allergic with the desensitized, in general, in the latter the lesions were harder and showed less necrosis than in the former. Many of the lesions of the desensitized group were of the compact epithelioid cell variety. Only the two worst cases among the desensitized group showed fresh, soft, apparently rapidly spreading lesions.

In many of the immune animals occasional minute healed hyalinized lesions were found, most commonly in the spleen. These were interpreted as being due, in part at least, to the immunizing R 1 infection, which frequently causes such lesions in this organ.

(*c*) *Skin lesions*

1. Clinical

Forty-eight hours after the inoculation, the *normal controls*, as noted in table IV and fig. 1, showed practically no reaction at the site of injection. About one week later, however, an inflamed indurated swelling developed at this point. This swelling rapidly developed a necrotic center and, three weeks after the inoculation, all of the animals of this group had sloughing ulcers varying from 3 to 8 mm. in diameter. These ulcers repeatedly became crusted and then broke down again. In no instance did they heal externally up to the time of death of the animal.

The allergic animals, whether treated with glycerine or untreated, showed an acute initial inflammatory reaction which manifested itself by an area of erythema within 24 hours. Forty-eight hours after the injection the reddened area was indurated and in most instances showed a central slough. One week later the slough had been discharged and was replaced by a small crust. In many instances the lesion later healed externally and only a small area of induration remained. In other animals, discharging ulcers persisted but they were, on the whole, much smaller than those in the non-immune animals.

The desensitized animals showed no more initial allergic response than did the *normal controls*. Subsequently, some followed the course of the allergic animals, developing small crusted lesions, while others showed no external lesions at all. In the latter, a small area of induration could usually be felt.

2. Pathological

At autopsy, the skin of the back of the neck was removed from each animal and spread flat on a piece of perforated cardboard. After fixation, sections were made through the lesion, if it was grossly recognisable, or from three adjacent blocks of tissue cut to include the region where the lesion should have been. In addition one animal of each group was sacrificed each day for the first three days after inoculation for histological study of the skin lesions. Subsequently, the skin specimens were obtained from time to time at more irregular intervals.

It is to be remembered that the skin on the dorsum of the neck is unusually thick. In most guinea-pigs the corium here attains a thickness of about 2 mm. This was one of the reasons why this region was chosen as the site of the intradermal inoculation, for it was possible here to introduce the whole inoculum (0.1 cc.) into the corium with minimal danger of accidentally puncturing the subcutaneous tissues. In this way we were assured against an accidental, traumatic spread of the infection, and could expect that the tissues which were later to be examined were all subjected to an initially comparable concentration of organisms.

Twenty-four hours after the inoculation, the *allergic* animals showed a diffuse oedema of the corium with a severe and relatively wide-spread infiltration consisting mainly of lymphocytes and polymorphonuclear leucocytes. Forty-eight hours after the inoculation, the central area showed liquefaction and abscess formation, while in the periphery epithelioid cells were accumulating in increasing numbers (fig. 3).

In contrast to these, the *desensitized* animals showed no oedema during the first two days and only insignificant cellular infiltration, mainly epithelioid cells and lymphocytes. The initial reaction of the *normal controls* was indistinguishable from that of the desensitized animals.

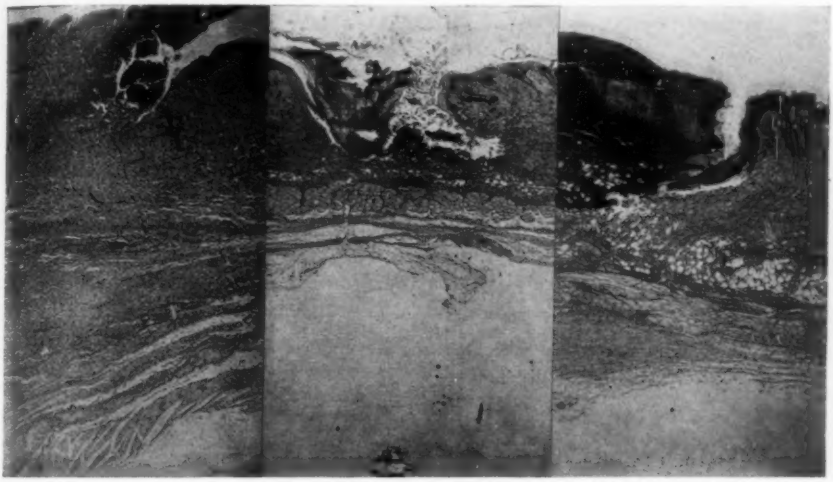
Between one and two weeks after inoculation, specimens of skin obtained from 4 animals showed the following reactions:

(1) *Immune allergic:* Diffuse oedema and round-cell infiltration of the subepithelial layers with large conglomerate tubercles in the deeper layers of the corium, some of which show central necrosis.

(2) *Glycerine control:* Diffuse round-cell infiltration of the whole

corium with confluent tubercles in the deeper layers extending down into the subcutaneous fat. Beginning necrosis in some of the tubercles.

(3) *Desensitized:* No oedema. Slight diffuse round-cell infiltration in the subepithelial layers with one solid epithelioid cell tubercle, without necrosis.



A B C

FIG. 4. Skin reaction 7 weeks after intradermal inoculation of virulent bacilli: A. Normal Control showing necrosis of the whole thickness of the cutis and subcutis with tuberculous infiltration extending into the deeper tissues; B. Desensitized showing a sharply walled off ulcer in the cutis without any appreciable reaction in the subcutis; C. Immune allergic showing necrosis of the whole thickness of the cutis with infiltration and caseation in the subcutis.

(4) *Normal control:* Some oedema of the corium with diffuse round-cell and epithelioid-cell infiltration extending through the whole corium and into the subcutaneous fat. No definite tubercles seen.

The following *summary* may be given of the general course of the infection in the skin:

The reaction in the *normal animals* was characterized in the later stages by the presence of large ulcers and intracutaneous abscesses, and the continuous spread of the tuberculous infiltration into the subcutaneous tissues (fig. 4).

The *immune allergic animals* showed in the later stages less widespread lesions than the normals. Tubercle formation within the corium was more prominent. Spread to the subcutaneous fat was less frequent. The frequency of recurrent ulceration and healing in this group has already been noted. Most of the lesions four or more weeks old showed caseation. No difference was noted between the untreated allergic animals and those receiving glycerine.

The *desensitized animals* showed less extensive lesions than did the *allergic*, less tendency to caseation, and decidedly less tendency to spread into the subcutaneous fat. In table VIII, the data are presented on 32 individuals selected at random from among those in which the lesion was more than one week old at the time of autopsy and

TABLE VIII

Skin lesions following intradermal injection of living virulent bacilli

Summary of cases in which the injection was more than one week old.

GROUP	NUMBER OF ANIMALS	LESION LIMITED TO THE CORIUM	LESION EXTENDING INTO SUBCUTANEOUS FAT
Normal Controls	7	0	7
Immune Allergic	6	1	5
Glycerine Controls	9	1	8
Immune Desensitized	10	7	3

which were studied with special reference to the spread of the infection from the corium to the subcutaneous tissues. In addition to exhibiting this greater power of local fixation of the infection in the tissues into which the inoculation was made, the desensitized animals showed considerably more tendency to form a fibrous capsule about the dermal lesion than did the allergic animals. Caseation occurred but rarely in this group.

In summary, the study of the effects of intradermal inoculation of living virulent tubercle bacilli in these groups of animals showed no evidence of allergy in the desensitized animals but classical signs of allergy in both the untreated and the glycerine-treated allergic groups. The general course of the skin infection was less severe in the desensitized than in the allergic animals. Far from losing the power to fix the infection locally at the site of infection, the desensitized animals

appeared to show this ability to a greater degree than did their allergic controls.

(*d*) *Ocular lesions*

The injections were made into the eyes primarily to enable us to follow minutely the severity of a visible focal lesion during the life of the guinea-pig. Whereas a small amount of inflammation may be present without producing macroscopic reddening or oedema of the skin, a similar amount of inflammation can less readily escape detection in the eye. The infected eyes were examined, with a hand slit lamp and a binocular loupe daily during the first week after the inoculation and afterwards every other day or twice a week. In this way one can observe not only the slightest congestion of the vessels of the conjunctiva and iris, but also minimal change in the transparency of the aqueous and cornea resulting from inflammatory exudation. Now it is obviously impossible to examine one hundred eyes daily and preserve in one's mind any clear notion of the relative severity of the reactions of the various groups into which they are divided. Some system of examination and classification is necessary. Each symptom of the ocular infection was arbitrarily graded into three or four degrees of severity. Records were thus made as to the degrees of the following symptoms: conjunctival congestion, conjunctival oedema, congestion of iris, exudation or tubercle formation on iris, constriction of pupil, exudation into pupil, turbidity of aqueous, opacity of cornea, and vascularization of cornea. The relative prominence of different symptoms in different groups could thus be gauged, or the total severity indicated by the sum of all the marks for all the symptoms. The latter number could be averaged for each group. Again it must be emphasized that the figures so obtained are not to be taken as rigidly quantitative. If the record for one eye is given in a figure twice as great as that of another, it is to be taken as indicating merely that the former was worse than the latter, not that it was twice as bad. Table IX illustrates the type of record obtained on a single guinea-pig's eye which, at the end, showed a confluent caseous lesion of the iris, ciliary body, and cornea.

In order that the results tabulated by such a system should have validity, it is necessary that the symptoms chosen and the arbitrary

grades decided upon should have been judiciously chosen and decided upon. This was determined experimentally by applying the scheme to sets of animals with varying immunity or varying doses of intraocular infection. It was possible in this way to test whether this method of measuring the severity of the lesion would give a steadily rising figure in the case of an obviously steadily progressing lesion, whether a more severe infection caused by a larger initial infecting dose would give a more rapidly rising figure, and finally, whether similar curves could be obtained in similar experiments. In the search

TABLE IX

Specimen of record of ocular lesions

Guinea Pig #18–22, Immune Allergic

DATE	CONJUNCTIVAL CONGESTION	CONJUNCTIVAL EDEMA	IRIS CONGESTION	IRIS EXUDATE	PUPIL CONSTRICTION	PUPIL EXUDATE	CORNEAL OPACITY	AQUEOUS TURBIDITY	TOTAL
4/22	2	2	0	0	0	0	1	0	5
23	1	1	0	0	0	0	0	0	2
25	0	0	0	0	0	0	1	0	1
27	0	1	0	0	0	0	0	0	1
29	1	1	0	0	0	0	1	0	3
5/2	1	1	0	0	0	0	1	0	3
4	1	1	0	1	0	0	2	0	5
6	2	3	0	1	0	0	3	0	9
9	2	2	1	2	0	0	3	0	10
13	2	3	2	2	1	0	5	1	16
16	1	2	1	2	2	0	5	1	14
20	3	3	2	3	2	2	6	2	23

for these criteria, records were made by this method on four sets of *immune allergic* animals inoculated with different doses of virulent bacteria, on two sets of *normal* animals inoculated with different doses of bacteria, and on two sets of *immune allergic* animals inoculated with the same dose of bacteria at the same time. The results are presented graphically in chart 2. Each curve represents the average reading on 6 to 12 guinea-pigs. These curves show that the method of measurement which we have outlined is in fact valid by the criteria enumerated above.

Turning now to the results of the intraocular inoculation in our major experiments, we find that in all three of the series in which this

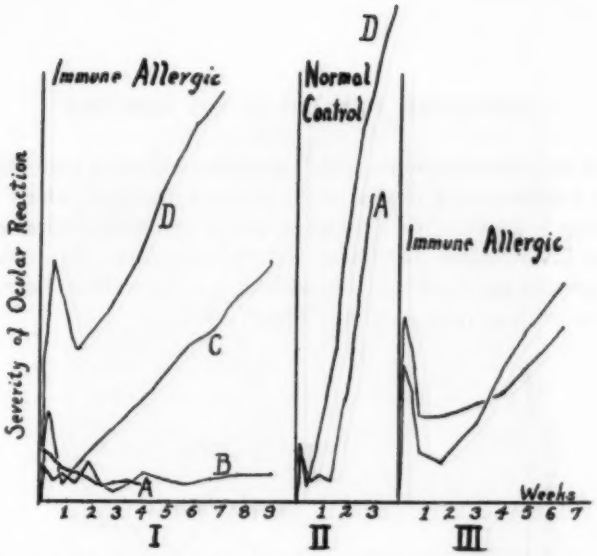


CHART 2. Charts showing the results of tests as to the reliability of the proposed method of gauging the severity of the ocular reactions following inoculation. Each curve represents the average readings on six or more animals.

I. Four groups of *immune allergic animals* all inoculated under similar conditions with the same immunizing dose of avirulent bacilli, subsequently inoculated with different numbers of virulent tubercle bacilli into the anterior chamber of one eye.

A. Three weeks' growth of bacilli on one Petroff slant emulsified in 10 cc. physiological salt solution, filtered through fine-grained filter paper; 0.005 cc. containing approximately 25,000 bacilli, none in clumps, injected intraocularly. No reaction.

B. Same emulsion as in A, filtered through coarse filter paper, diluted 10 times and refiltered; 0.005 cc. containing approximately 1000 organisms, some in clumps, injected intraocularly. Slight initial allergic reaction, no progressive tuberculosis.

C. Same emulsion as in A, filtered through coarse filter paper; 0.005 cc. containing approximately 50,000 bacilli, many in clumps, injected intraocularly. Moderate initial allergic reaction with complete recovery followed by a steadily progressive lesion.

D. Same emulsion as in A, unfiltered,—milky suspension. Severe initial allergic reaction which is followed, without intervening recovery, by a severe progressive lesion.

II. Two groups of *normal, non-immune animals* receiving doses A and D, respectively. Except for a longer incubation period, there is no significant difference in the reaction to the smallest as compared with the largest dosage of bacilli.

III. Two groups of *immune allergic* animals infected at the same time with the same dosage of bacilli, approximately equal to dose C of I. Severe initial allergic reaction followed by a progressive lesion. The severity of the progressive lesion is the same as in C of I, but the initial allergic reaction more severe. This might be interpreted as signifying that these animals were more allergic but equally immune as compared with those of I, but it is equally possible that larger fragments of the culture medium containing more tuberculoprotein were included in the emulsion. This illustrates the necessity of inoculating all animals in one experiment with the same emulsion of bacilli at the same time if comparable results are desired.

type of measurement was applied, comparable results were obtained. Chart 3 presents the results in the fourth experiment which we are reporting in detail. The findings of special ophthalmological interest in this investigation have been reported elsewhere (13). Here we shall present the results only in summary so far as they bear on the problem of allergy and immunity (figs. 5 and 6).

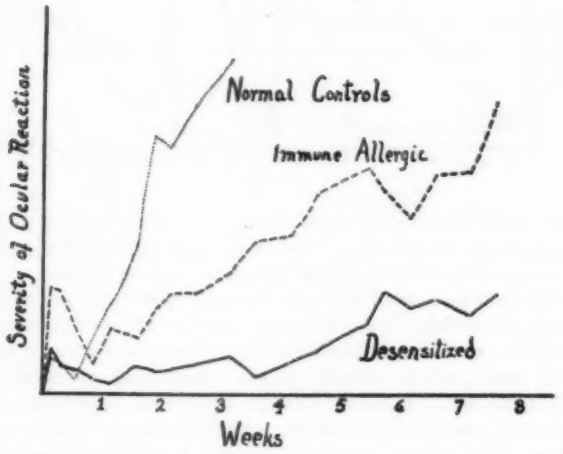


CHART 3. Chart showing the severity of the ocular reactions in the final experiment.

1. Normal controls

The eyes of normal non-tuberculous animals inoculated with tubercle bacilli into the anterior chamber showed during the first two days a very slight reaction referable to the trauma of the injection. Following this they became entirely clear and for a week or more no clinical evidence of the infection was visible. During the second week after the injection, conjunctival congestion reappeared and small yellowish white tubercles were visible in the iris. The lesion progressed with great rapidity and by the fourth week the great majority of these eyes showed complete caseation of their whole anterior segment. Subsequently, the eyeballs ruptured and discharged their caseous contents.

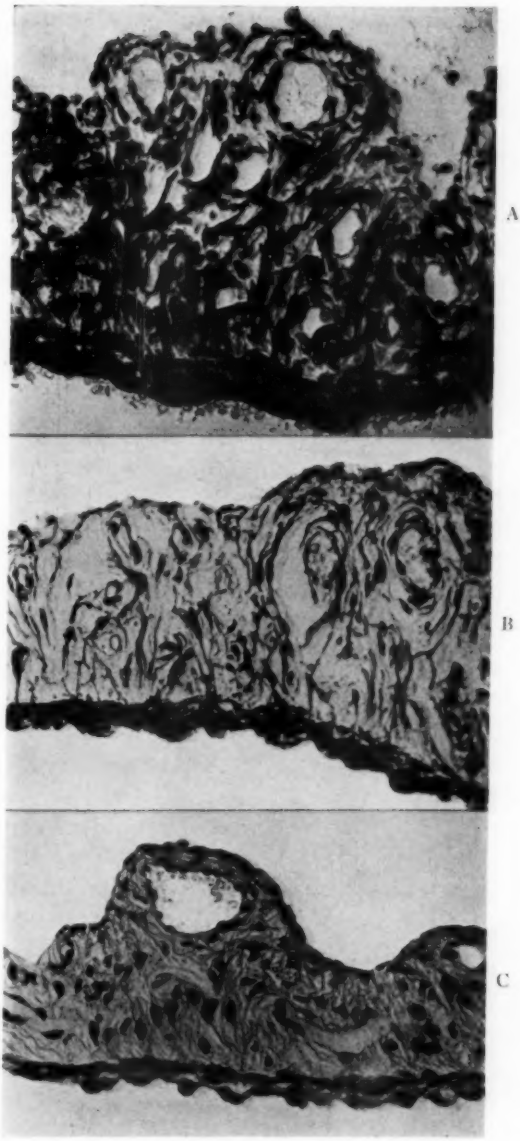


FIG. 5. Sections of iris 48 hours after intraocular inoculation of virulent bacilli: A. Immune Allergic; B. Desensitized; C. Normal Control.

A B C

FIG. 6. Eyes 5 weeks after inoculation with virulent bacilli: A. Immune Allergic; B. Desensitized; C. Normal Control.

2. Immune allergic

During the first two days following intra-ocular inoculations, the eyes of the *immune allergic* animals showed an intense acute inflammatory reaction with marked conjunctival congestion and oedema, diffuse clouding of the cornea, turbidity of the aqueous often with the formation of fibrin clot in the aqueous, constriction of the pupil, congestion of the iris. The character of this acute allergic reaction in the eye could be markedly influenced by the dosage and state of aggregation of the organisms injected. If careful precautions were taken to avoid clumping of the organisms, a fairly large dose of bacilli (25,000 organisms) could be given without producing any detectable allergic reaction. If clumps of bacilli were present, the allergic reaction developed, and the severity and duration of the reaction was found to vary with the dosage of the infection. The dosage chosen for the series which we are reporting was such that a definite initial allergic reaction was produced, but one after which the eyes returned to a normal state in which no clinical signs of infection were visible. This seemed to us important in order to avoid the possible criticism that the severity of the subsequent progressive lesion was in part due to residua of the initial allergic response.

After an interval of several weeks during which these eyes were entirely free from symptoms, a slowly progressive reaction developed which was in all respects similar to the type of tuberculous keratoiritis familiar to ophthalmologists. The progress of the lesion was much slower than that in the normal controls and only a few of these cases reached the stage of complete caseation of the anterior segment of the eyeball. An equally small number showed no progressive ocular disease within the period of the experiment.

3. Glycerine controls

The ocular reaction of this series of animals was in all respects comparable to that of the immune allergic group, except that they were slightly more severe. Apparently the daily subcutaneous injections of glycerine and the consequent susceptibility of these animals to intercurrent disease exerted only a slightly deleterious effect on the course of their local ocular lesions.

4. Immune desensitized

These animals failed completely to show the initial allergic reaction which characterized the early stages of the ocular lesions in the two preceding groups. During the first few days following the inoculation, these animals showed no more ocular reaction than did the *normal controls*. This finding contributes additional evidence of the degree of desensitization which had been achieved. After an interval of several weeks, a slowly progressing ocular lesion appeared in some of these animals. The clinical characteristics of the affection were similar to those of the *immune allergic* animals but were decidedly less severe and progressed much more slowly. No case in this group reached the stage of complete caseation, and a large number showed no clinically demonstrable ocular lesions (fig. 6).

5. Histological findings

All the infected eyes were studied histologically. In no case were any lesions found which did not present the classical features of tuberculosis. This is important, since there has been a tendency among ophthalmologists to believe that tuberculosis in the eye may take on histological characteristics unlike those which the disease produces in other organs. During the acute allergic reaction, an exudation of polymorphonuclear leucocytes, lymphocytes, and epithelioid cells was found in the anterior chamber with similar infiltrates in the surrounding tissues. The iris and cornea were oedematous, the vessels of the iris and conjunctiva congested. The picture was, therefore, entirely analogous to the acute allergic reaction to tubercle bacilli with which pathologists are familiar and which we found likewise in the skin following the local injection of a suspension of living bacteria. The eyes of normal and of desensitized animals examined at the same stage following the infection showed only a slight increase in the protein content of the aqueous (fig. 5).

The progressive tuberculosis of the eyes, when it appeared, showed the same histological characteristics as that of the other organs, and the same differences already noted between the soft caseating lesions of the normals and the sluggish harder lesions of the immunes. The desensitized animals again showed the smallest, hardest lesions and the least tendency to necrosis.

6. Summary of ocular reactions

Summarizing the findings on the eyes, it may be noted that they are entirely in accord with those in the other organs. There is no evidence that the loss of allergy deprives the immunized animal of any aspect of his immunity so far as his ability to combat the progress of local lesions is concerned. On the contrary, the local ocular lesions in the desensitized animals were more circumscribed, less severe, and less rapidly progressive than those in the immune allergic animals.

(*e*) *Mortality*

The analysis of the mortality rates in these different groups of animals presents considerable difficulties. As noted above, the animals receiving tuberculin showed a high mortality, even before inoculation with virulent tubercle bacilli. Careful examination of the organs of the desensitized animals which died before receiving a virulent infection failed to reveal any evidence of progressive tuberculosis. The mortality during this period cannot, therefore, be attributed to any effect which the injections may have had upon the reaction of the host to the avirulent R 1 infection. Different animals develop a different degree of hypersensitivity following infection, and it is well known that the hypersensitivity of different individuals is not reduced to the same degree by desensitizing injections of antigen; and since our daily doses of tuberculin were the same for all of the animals which we were attempting to desensitize, it was to be expected that not all of the animals under treatment would tolerate in the same manner the dose administered. In these experiments, it was obviously impossible for us to determine by graded tuberculin tests the degree of sensitivity of each individual animal each day and to increase the dose of tuberculin in each case according to the individual hypersensitivity. A similar mass treatment of a group of human beings by means of a uniform, increasing dose for all would, it is well known, lead to severe reactions and death of some of them because of differences in the degree of hypersensitivity. After the treated group had reached the dosage of 2000 mgm. of tuberculin, the degree of mortality declined, in spite of (or because of) the fact that they were receiving much larger doses of tuberculin. This, at least, indicates

that the higher mortality earlier in the experiment was not due to any primary toxicity of tuberculin and nothing is known at present of any toxin in tuberculin which is neutralizable by antitoxin. At any rate, the facts justify the conclusion that the mortality from tuberculosis in the tuberculin-treated group during the period of observation was zero. This conclusion was confirmed by the pathological examination of the organs of these animals. None of this group showed lesions that could be classified as massive or even as severe.

The mortality of the broth-treated animals during the period prior to virulent infection was high, that of the glycerine controls during this period was less than that of the broth- or tuberculin-treated group, and that of the immune allergic untreated animals considerable less than either of the others. After virulent infection, the glycerine control group showed a slowly rising mortality rate, while that of the immune allergic untreated group remained at its previous low level. While the numbers involved are too small to warrant rigid statistical analysis, the pathological findings that some of these animals showed severe and even massive tuberculosis are sufficient evidence that they died of tuberculosis.

The normal control group showed only a minimal mortality prior to infection. Deaths in this group reached a maximal incidence in the fifth week following infection and were associated with massive visceral tuberculous lesions.

IX. REMARK

It cannot be too strongly emphasized that the dosage of tuberculin used to achieve complete desensitization of the guinea-pigs in the present experiments was relatively enormous, and we are not by any means to be understood as suggesting that a similar procedure and proportional doses should be applied in human cases. The reports of therapeutic success in the treatment of tuberculosis by tuberculin, especially ocular tuberculosis, may, however, be interpreted on the basis of our findings as being due to a perifocal desensitization rather than, as has been heretofore assumed, to minimal perifocal allergic reactions. Our continual and direct study of the ocular lesions by methods which permit any inflammatory reaction to be seen make it clear that the beneficial results of tuberculin in the present experiments

were associated not with mild allergic reactions but rather with no reactions at all (desensitization). In a patient suffering from tuberculous infection, the degree of allergy is certainly not uniformly distributed throughout all the tissues. It has long been known that the degree of allergy in the tissues at the site of a tuberculous focus is far in excess of that in the rest of the body. It is quite possible, therefore, that a careful graded course of injections of tuberculin far below the level of the massive doses used in this experiment may be sufficient to diminish the perifocal allergy of the tissues and so to produce therapeutically beneficial results. It must be pointed out, however, that we are as yet totally in ignorance of those factors which determine the narrow zone within which the dosage of tuberculin is effectively desensitizing, and beyond which it is either too small to produce any effect, or so large as to produce deleterious perifocal reactions. The rational and vigorous pursuit of therapeutic desensitization must await the discovery of some effective non-specific desensitizing agent,—an agent which produces desensitization but which is free from the danger of producing deleterious allergic reactions. None of the many non-specific desensitizing agents which has heretofore been described can be used with anything like constant effects.

CONCLUSIONS

1. Complete desensitization to tubercle bacilli and to tuberculin can be achieved in tuberculous guinea-pigs by a prolonged and properly graded course of subcutaneous injections of Koch's Old Tuberculin. The desensitizing power of purified tuberculo-protein is less in proportion to its power to produce allergic reactions than is that of Koch's Old Tuberculin.

2. Long continued daily subcutaneous injections of massive doses of concentrated glycerine broth in some instances desensitizes tuberculous guinea-pigs to tuberculin. This non-specific desensitization is not due to the glycerine contained in the broth. It is not known whether this non-specific desensitization is free from the danger of perifocal reaction.

3. Infection in desensitized immune animals does not introduce into the histological picture of tuberculous lesions features that are novel to the pathology of human tuberculosis.

4. Complete desensitization does not deprive the treated animals of any aspect of their immunity as measured by the course and severity of local lesions, by the spread and severity of their visceral lesions, and by the mortality from tuberculosis in infected animals. In all these respects the reaction of the desensitized animals was equal to or superior to that of the non-desensitized controls. In so far as the inhibition of spread of lesions from the site of infection to the viscera may be used as evidence of local fixation of bacilli, the desensitized, non-allergic immunes were able to resist the spread of infection as successfully as the allergic immunes.

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A STUDY OF THE CHARACTER AND DEGREE OF PROTECTION AFFORDED BY THE IMMUNE STATE INDEPENDENTLY OF THE LEUCOCYTES

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The important rôle played by the phagocytic leucocytes in both natural and acquired immunity has long been definitely established, but it is still a matter of question whether various humoral alterations which occur during immunization are, in themselves and independently of the phagocytes, agents which are able to protect the body against bacterial invasion and, if they do so serve the body as protective agents, it remains to be determined in just what manner their presence aids in combating infection. The protective value of opsonin is obvious, but this virtue is entirely dependent upon the coöperation of the phagocytes and cannot, therefore, be regarded as a purely humoral mechanism of defense. It is only in rare instances that acquired antibodies which are potent in destroying bacteria without the coöperation of the phagocytes have been demonstrated. The phenomena of agglutination, precipitation and complement fixation are very common accompaniments of immunity, but since survival immunity may exist in the absence of detectable amounts of the antibody responsible for the operation of these phenomena it is not uncommon to meet the statements that these processes bear no constant relation to immunity to infection, that immunity is not dependent upon their existence, and that their precise rôle in immunity if, indeed, they play one, is undetermined.

Omitting the effects of antitoxins from this discussion, a highly developed state of acquired immunity exhibits itself in two important ways. In the first place, whereas virulent, invasive bacteria begin to spread almost instantly from the site where they lodge in the tissues

of the non-immune body, they are held fixed at the site, often to a remarkable degree, in the immune one and the spread of infection to distant tissues is thereby inhibited or even completely prevented. In the second place, in the immune body the growth of the invading bacteria is suppressed, most or all of them are destroyed, and the body survives the infection.

Different opinions exist regarding the exact means by which these two important manifestations of immunity—the local immobilization of the bacteria and the suppression of their growth—are effected. Practically all writers have ascribed the remarkable prevention of spread of bacteria in the immune body purely to the mechanical, walling-off effect of the inflammation which appears about them. Now it has been well known for forty years that when inflammation is once established it can inhibit the spread of bacteria even in the non-immune body. However, since the time required for an effective amount of inflammation to appear about invasive bacteria, even in the allergic body in which the process is accelerated, has been found by all who have investigated the matter to be distinctly longer than the time required for bacteria to spread from the site at which they lodge (1), (2), (3), (4), (5), (7), (27) we have never been able to accept the view that it is inflammation which is *primarily* responsible for preventing their immediate spread in the immune body, and one of us has, in another place (8), demonstrated the rôle which immune antibody plays in immobilizing pneumococci during the period before inflammation is well enough established to assist in preventing their spread. It will be seen that the experiments to be presented in this paper have served to expose this protective action of the antibody in a further and very striking way. As for the suppression of growth of the bacteria, although the importance of the activity of the phagocytes in this connection is now thoroughly well appreciated, some have believed that, in addition, the fluids of the immune body may possess acquired bactericidal properties which may be operative *in vivo* even though they cannot be demonstrated *in vitro*, and it has also been suggested that the immune body has undergone some more subtle change which renders it less favorable than the normal body as a medium for the growth of the bacteria. Much has been written also about the possibility of the existence of a "tissue immunity," often with the

implication that in the immune body the fixed tissue cells themselves have, in some obscure way, become immune to infection.

We felt that some light might be thrown upon the question of whether acquired immunity confers upon the body any power of resisting infection which operates independently of the phagocytes, by studying the course of infection in highly immunized animals possessing an abundance of antibody, but in which the factors of phagocytosis and the pouring out of purulent exudates about the bacteria were eliminated. We have been able to find in the literature only one report in which immunized animals were rendered leucopenic and their ability to survive infection then studied. In those experiments, which were carried out by Winternitz and Kline (9), pneumococcal infection was studied in immunized animals treated with benzol to destroy their leucocytes. The authors reached the conclusion that there exists in the body with acquired immunity a mechanism of some undedetermined nature which effectively destroys pneumococci independently of the activity of the phagocytic leucocytes, for their actively immunized, benzol-treated animals survived the test infection.

"When actively immunized, benzolized animals are injected with a lethal dose of pneumococci . . . . the ultimate reaction differs from that in the passively immunized aplastic animal. The latter develops a recurrent bacteremia and dies. The actively immunized animal recovers. It would seem that the white blood cell is no longer necessary in an animal that has been actively immunized," they write.

Certainly, the report of these careful investigators shows plainly that their actively immunized, leucopenic animals were able to survive the infection under the conditions of their experiments. There are, however, several circumstances which render their experiments an insufficient test of the conclusion drawn from them, and it will be seen from the experiments to be reported in the present paper that the phagocytes are essential for immunity to the Type I pneumococcus, and that the immune body possesses no other means of preventing their progressive growth. In the discussion of these experiments the reasons for the conflicting conclusions of Winternitz and Kline and of ourselves will be pointed out.

EXPERIMENTAL METHOD

In the present experiments we have studied the course of Type I pneumococcal infection in a total of one hundred and thirty-one, animals. Of these, forty-six were immunized, benzol-treated rabbits, fifty-four were immunized controls not treated with benzol, seven were non-immune, benzol-treated rabbits, and twenty-four were normal rabbits which served to check the maintenance of virulence of the cultures used during the course of the experiments. The skin was chosen as the site of infection for the reason that the common procedure of intravenous inoculation for testing immunity provides no opportunity for studying the fate of bacteria lodging in the tissues. It is clear that intravenous infection precludes the study both of the local fixation of bacteria and of the mechanism of bacterial destruction within the tissues, phenomena which, excluding the action of antitoxin, constitute the most important events in the operation of immunity under the conditions of natural infection. Furthermore, through previous studies (8), (12), (13), we had become thoroughly familiar with the gross and microscopic reactions following intradermal infection with the pneumococcus in both normal and immunized rabbits, and had found, in common with Goodner (14), that the skin is an exceptionally favorable tissue for the study of the progress and retrogression of local infection with this bacterium. The progress of cutaneous lesions can be watched macroscopically and the sites can be excised for microscopic study at any desired stage of the infection. The injection was made on the upper part of the animal's side and when sections of the lesions in the earlier stages of the infection were desired, it was our practice to inject each side of the animal with the same dose of pneumococci and to remove one of the lesions at the desired time, fixing it immediately in Zenker-formol, and leaving the lesion on the opposite side to serve as a test of the animal's resistance to the infection.

The culture of pneumococcus (Type I) which we used was of a virulence such that the intracutaneous injection of 0.1 c.c. of a 1:1,000,000 dilution of a blood broth culture would produce in normal rabbits a large, progressively spreading, oedematous and haemorrhagic lesion accompanied by septicaemia, and leading always to death in less than sixty hours. The amounts of culture used for infecting the

immunized animals (0.1 c.c. of 1:10, 1:100 or 1:1,000 dilution) always killed normal, non-immune animals within twenty-four to thirty-six hours. Tests of the virulence of the culture for normal rabbits were made repeatedly during the course of the experiments.

In order to render the animals leucopenic we made use of benzol[1] which, since the early studies of Selling (10), has been recognized to be a powerful and specific leucocytotoxin. Hamilton (11) has recently published an excellent review of the literature on the experimental use of benzol for the purpose of destroying the leucocytes in the study of a wide variety of physiological, pathological and immunological problems. In our experiments we followed roughly the method of Selling, who recommended the daily subcutaneous injection of 1 c.c. of benzol per kilogram, the benzol being mixed with an equal quantity of olive oil. Since our rabbits averaged about 2,000 grams in weight, we injected a standard daily dose of 4 c.c. of equal parts of benzol and olive oil beneath the skin of the abdomen until the leucocytes had dropped to 250 or less per cubic millimetre, at which point we proceded with the test infection, for we had found by preliminary experimentation that when the circulating leucocytes were kept at or below 250 per cubic millimetre, ordinarily no cells at all would migrate from the blood into the tissues at the site of infection, regardless of the number of pneumococci present or of the degree of tissue damage. Under such conditions it is possible to follow minutely the course of infection and the fate of the bacteria in leucocyte-free lesions. Ordinarily it required from five to seven days to effect the depression of the leucocytes to the desired degree, but it should be stated that it is not possible to bring the leucocytes to this level in all animals, nor is it possible, when that point has been reached, to maintain them there at will without the occurrence of a further disastrous fall, or of an unexpected sudden rise. If the white count drops below 100 the animal ordinarily dies rapidly, even though the administration of benzol has been discontinued; and, on the other hand, even under the continued daily administration of benzol, a sudden rise in the number of leucocytes may occur after the desired level has been attained. Thus, if the test infection is given to three animals on the day on which their leucocytes have reached the level of 250 per cubic-millimetre, one of the animals may maintain this level or fall only slightly

[1] The preparation used was "Benzole C. P.", Muth Brothers, Baltimore, Md.

below it during the several days necessary for the complete study of the course of the infection; in another the leucocytes may rapidly drop to zero and the animal will die before there is time for the infection to run its course; and in the third the leucocytes may rise rapidly to the level of seven or eight hundred and the experiment will be rendered useless because of the emigration of leucocytes which always occurs at the site of infection when the white count is that high. At best, the use of benzol for the purpose of obtaining leucocyte-free lesions is a capricious method, for different animals react very differently to the same dose by weight of benzol, and furthermore it is impossible to forecast the degree of leucocytic depression which will occur from one day to the next in any particular animal under a given dosage of the drug. In this work, therefore, many animals have been rendered useless for the purposes of our study by uncontrollable variations in their white count during the period of the test infection. It is furthermore obvious, that not all of a series of animals in which treatment with benzol is begun at the same time will be ready for the test infection at the same time. It is therefore necessary to be continually prepared to use any of the animals of a series as soon as their leucocytes reach the desired level. However, in spite of the difficulties which arise from the impossibility of conveniently standardizing the method of procedure, one can, by repeated trials, amass an entirely satisfactory group of experiments in which the leucocytes are brought to the desired level and maintained within the bounds necessary for the production of leucocyte-free lesions during a period of time sufficient for the course of the test infection to be studied as desired.

Many of the animals bore the benzol treatment with surprisingly little loss of weight. In others, the appetite diminished and they lost weight progressively. As a control for this factor of undernutrition we starved immunized animals, which were not treated with benzol, for the same length of time as that during which benzolized animals were under treatment, and then infected them with the same dose of the same culture of pneumococci. The immunity of these animals, as determined by the course of infection, character of lesions and absence of septicaemia, was in no way affected by the period of complete starvation. Infection in these animals differed in no detectable way from that in well-fed immune animals.

Active immunization of our animals was accomplished by means of repeated intravenous injections of concentrated heat-killed cultures of virulent Type I pneumococci, carried out over a period of a number of weeks. When the rabbits of a given series had received fifteen or more intravenous injections of 1 c.c. of a heavy suspension of dead organisms at intervals of several days, it was determined by a test intradermal inoculation with living virulent pneumococci that they were able to survive at least one hundred to one thousand times the infecting dose to be used in the experiments. Then half of the animals were treated with benzol as described above, and leucocyte counts were made daily. As soon as the leucocytes of any of the animals had been reduced to about 250 per cubic millimetre, the test for immunity in those animals was carried out as follows: A large area, well removed from the site of the benzol injections, was carefully freed of hair by shaving or by means of a depilatory. 0.1 c.c. of an eight to twelve-hour growth of the virulent culture described above, diluted to the desired degree (1:1,000, 1:100 or 1:10) was then injected intradermally into the immunized benzol-treated animals. At the same time the same dose of the same culture was injected intradermally into rabbits of the group which had been immunized but had not been treated with benzol. These animals served as immune controls for the benzol-treated group, so that the course of infection in immune animals possessing leucocytes could be compared with that in their immunized mates in which the participation of the leucocytic defense mechanism was eliminated.

We have carried out numerous agglutination tests during the course of these experiments and we have found no appreciable alteration of the agglutination titre produced by the administration of benzol. This has been the experience of others who have studied the effect of benzol upon an established antibody titre (21). Furthermore, there was no appreciable diminution in the amount of complement. However, we wished to have a control which would demonstrate directly that the benzol-treated animals possessed at the time of the test infection a concentration of antibody which would have been amply sufficient to protect them against infection had they also possessed leucocytes, so we took pains to demonstrate the protective power of their serum by the method of passive transfer. 4 to 8 c.c. of serum

taken from each benzol-treated rabbit on the day of the test infection were injected intravenously into normal, non-immune rabbits and these latter animals were then infected with the same dose of culture as that which the benzol-treated animals received.

In addition to this control for the demonstration of the efficacy of the serum of the benzol-treated rabbits in passive protection, a second form of passive immunity test was carried out. This latter method consisted in making up a dilution of the culture to the same degree as that given to the benzol-treated animals, but instead of broth as the diluent, serum from the individual benzol-treated animals was used for the final dilution. When the dilution of culture had been made in broth up to the point at which a final dilution of one to ten would yield the ultimate dilution desired, 0.1 c.c. of the diluted culture was added to 0.9 c.c. of serum from the benzol-treated animal which was to be tested. Thus, if 0.1 c.c. of culture diluted 1:100 in broth was the infecting dose given to a particular benzol-treated animal, 0.1 c.c. of the same culture diluted ten times in broth and then ten times in the serum of that animal was given intradermally to a normal, non-immune control. The injection was always given immediately after the dilution was made, before any agglutination had taken place. This form of passive protection experiment represents, in effect, the injection of "sensitized" bacteria into a normal animal and served to provide information regarding the power of the body fluids of the immunized, benzol-treated animals to affect the bacteria on a single exposure, for there was of course no possibility of a further replenishment of antibody by way of the tissue-fluid stream after the bacteria had reached the tissues of the normal animal. As a control for this series, we carried out experiments in which normal rabbits were infected with the same dose of culture diluted in normal rabbit serum. The course of infection in these latter animals was always precisely like that in the culture controls, which consisted of normal rabbits infected with the same dose of the same broth dilution of culture as that given to the immunized, benzol-treated animals.

Finally, non-immune rabbits treated with benzol were infected with the same dose of pneumococci as that given to the culture controls in order to determine whether the benzol treatment in itself exerted any effect upon the course of infection in non-immune animals.

Our experiments comprised, therefore, the following groups of animals, all infected intradermally with the same dose of pneumococci:

(1) Actively immunized, leucopenic animals.
(2) Non-immune leucopenic animals.
(3) Actively immunized animals with normal number of leucocytes.
(4) Animals passively immunized intravenously with serum of individual animals of group (1).
(5) Animals infected with pneumococci sensitized by serum of individual animals of group (1).
(6) Normal controls.

THE COURSE OF INFECTION IN ACTIVELY IMMUNIZED, LEUCOPENIC ANIMALS AND IN THEIR CONTROLS

In *normal controls* (group 6 above) intradermal infection with virulent Type I pneumococci in the doses used produces a lesion which becomes macroscopically definite as a pink, slightly oedematous area after the lapse of five to seven hours. With the passing of time the lesion spreads progressively over the side of the animal until, at the end of 24 hours there is a large, red, oedematous and often purpuric lesion extending from the top of the side, where the bacteria were injected, all the way to the under surface of the abdomen where the oedema is especially prominent. The blood culture becomes positive about five hours after infection and septicaemia is extremely heavy at the time of death. Microscopical study of lesions removed at different periods after infection and stained for bacteria show a gradual development of inflammation, which is well established five to seven hours after infection and which increases continually to a degree of great intensity. There is practically no phagocytosis of the bacteria by the abundant leucocytes. The pneumococci proliferate progressively from the beginning, and each pair of daughter cells separates from the others after division and drifts freely through the tissue spaces (figs. 6 and 8), the uninterrupted spread of the bacteria being indicated macroscopically by the continually advancing margin of the lesion.

In contrast, in the *actively immunized animals not treated with benzol* (group 3 above) the infection never spread progressively, but was always sharply limited to an area one to several centimetres in diameter which was pink and oedematous but never purpuric. This lesion

reached its height 24 to 48 hours after infection and then gradually regressed, to disappear completely in from five to ten days. The blood cultures remained sterile throughout, and the animals survived the infection. Microscopically, the bacteria were found to remain localized where they were injected, and they tended to grow in clumps adhering to each other and apparently to the tissues as well, until they were surrounded and engulfed by the leucocytes of the inflammatory exudate (fig. 7). Phagocytosis was easily seen in the sections. After twelve hours it was only by painstaking search that free pneumococci could be found, except when large doses were injected. With the passing of time the polymorphonuclear exudate was absorbed, mononuclear cells appeared in abundance, and dead tissue was eventually replaced by a scar.

The *actively immunized leucopenic animals* (group 1 above) constitute, of course, the group in which we are particularly interested. Macroscopically, the amount of oedema and inflammatory hyperaemia at the site of infection in these animals was always less than that in their immunized mates which had not been treated with benzol. At the end of 24 hours after infection some of the benzol-treated animals had lesions which, while of the same diameter as those of the untreated immunes, were rather less oedematous and hyperaemic. In others, the lesions were still less marked; and in a portion of the group no lesion whatever could be detected on macroscopic examination. With the further passing of time, however, lesions became plainly apparent even in the latter group. At 36 hours those lesions which were visible at 24 hours had become somewhat larger in diameter, and it was apparent that the bacteria were very slowly spreading through the tissues. In addition, purpuric spots were often present in the lesions. At this time in the animals which had had no visible macroscopic lesions at 24 hours the site of infection now appeared as a pale, slightly yellowish area, several centimetres in diameter. With the further passing of time the lesions in all of the animals increased in size slowly but steadily, until death occurred (fig. 1). The duration of survival after infection was between 48 and 78 hours. Blood cultures made daily or twice daily showed that the blood stream always remained sterile during the first 36 hours after infection. In a considerable number of the animals the blood culture was sterile at the end of 48 hours and in

one case it was still sterile at 55 hours. However, the blood culture eventually became positive in every benzol-treated animal which escaped the accident of death from benzol before sufficient time (two to three days) had elapsed for septicaemia to develop, or the accident of a sudden rise in the leucocyte count permitting the leucocytes to emigrate and to combat the infection.

The course of infection in the immunized animals with leucocyte-free lesions as compared with that in non-immune controls was characterized, therefore, by a marked retardation in the spread of the local lesion and a marked retardation in the development of fatal septicaemia. At the time when the lesions in the non-immune controls extended all the way from the vertebral column to the midline of the abdomen, those in the immunized leucopenic animals were far smaller, their diameter being no greater than those in the immunized animals possessing a normal number of leucocytes; but whereas the lesions in the latter remained stationary in size for a few days and then regressed and disappeared, those in the immunized leucopenic animals increased steadily in size, but so slowly that death with septicaemia ordinarily occurred before the lesions attained the size of those in the non-immune controls. In the absence of the leucocytic defense *survival immunity* was destroyed, but it was clear from the slow progress of the lesions and from the greatly delayed septicaemia that some factor of the immune state was still in operation delaying the fatal outcome. It is obvious that this might have been the result of either of two factors. On the one hand, it was possible that, even in the absence of the leucocytes, the immune state was in some way bringing about the destruction of many but not all of the bacteria in the lesions, thus delaying the fatal outcome; and on the other hand it was possible that the bacteria were not being destroyed, but that their spread from the site of infection was being inhibited by the process which one of us has described in another place (8),—a mechanism which operates independently of the leucocytes and which is effected by the antibody contained in the fluids of the immune body. Microscopical study of the fate of the bacteria in the lesions was the only direct method by which a solution of this problem could be obtained and the study of the lesions at once disclosed the fact that the longer survival of immunized, leucopenic animals as compared with non-immune controls

was the result of the operation of the mechanism of local immobilization and not of bacterial destruction. Sections of lesions removed at various intervals between 30 minutes after infection and the time of death showed that, just as is the case even in highly immunized animals possessing leucocytes, the bacteria begin to proliferate at once on reaching the tissues and adhere to one another after division, forming clumps (fig. 4). In the case of the immunized animal with leucocytes, the clumped, immobilized pneumococci are soon engulfed and destroyed by the phagocytes of the inflammatory exudate (fig. 7); but in those of the immunized, benzol-treated animals in which the circulating leucocytes remain at 250 per cubic millimetre or below, the sections show that in many cases not a leucocyte emigrates from the blood vessels at the site of infection, and in no case can one find more than an occasional leucocyte in the tissues (figs. 11 and 13). In some of the animals an outpouring of inflammatory oedema can be clearly seen; in some even this is too slight in amount to be detected in the sections. This absence of detectable inflammation in some of the benzol-treated animals, in spite of the presence of many bacteria, is a rather remarkable phenomenon. Camp and Baumgartner, likewise, studying the local effects of croton oil in benzol-treated rabbits write that "in many cases there is no reaction whatever in the leucopenic animals" (25). But in all of the animals of this group a steady multiplication of the bacteria proceeds uninterruptedly until the death of the animals. The bacteria are, however, held fixed at the precise spot where they were injected to a most remarkable degree for hours after those in the non-immune controls have spread through the tissues far from the site where they were injected and have invaded the blood stream. Thus, 24 hours after infection, at the time when the non-immune controls are dying or dead with heavy septicaemia and with huge lesions showing the presence of bacteria diffusely throughout an area 10 centimetres or more in diameter, the bacteria in the immunized, leucopenic animals are still confined to an area at most only a few millimetres larger than the area originally infiltrated by the injection of the 0.1 c.c. of fluid containing the bacteria. In contrast to the manner in which the daughter-cells of the pneumococci immediately separate and drift away from each other after division in the tissues of the non-immune body (figs. 5 and 10), they tend to remain adherent

to each other and, apparently, also to the cells and fibres of the tissues in the immunized leucopenic animals precisely as they do in immunized animals untreated with benzol (8). The result of this is that in the absence of the antagonistic leucocytes, the bacteria proliferate uninterruptedly, but they are held at the site; and their adherence to each other after division results in the formation of large, compact colonies of cocci which increase steadily in size (figs. 12, 14, 15) until at length a continuous felt-work of innumerable bacteria fills up the tissue spaces (fig. 13), the organisms finally penetrate into blood and lymph vessels and septicaemia becomes at length established. When only a few pneumococci escape into the blood stream they are, undoubtedly, rapidly removed by the well recognized increased clearing capacity of the blood, which results from the action of the immune antibody causing the bacteria to become agglutinated and to adhere to the walls of the capillaries, especially of the liver and spleen (20), and to be promptly ingested by the intravascular reticulo-endothelial phagocytes of those organs which, as we shall show in a moment, retain their power in this direction in benzol-treated animals. But when the proliferation of the bacteria in the local lesion has progressed to such an extent that great numbers are being discharged into the blood stream, the blood cultures remain continually positive. It is interesting that in smears of the blood of these animals made just before death, when septicaemia is well established, we have occasionally found pneumococci agglutinated in small clumps, in contrast to the invariably separate diplococci which are found in the blood smears of non-immune animals with septicaemia. Furthermore, it is important to state that the virulence of the bacteria was not altered by their residence in the immune leucopenic body during the period of the experiments. The pneumococci obtained from the blood when septicaemia was established, and at the time of death, were well encapsulated, they possessed the typical colony form and specific agglutinative characteristics of Type I pneumococci, and their virulence, as far as could be determined by the results of the injection of high dilutions of culture into non-immune rabbits, was the same as that of the pneumococci which were originally introduced.

In brief, then, highly immunized animals in which no leucocytes migrate to the site of infection invariably succumb to the infection,

but they survive for a considerably longer period of time than do non-immune animals possessing leucocytes; and the increased period of survival is the result of the action of the immune antibody which inhibits the spread of the bacteria through the tissues and into the blood stream, and which renders them susceptible to phagocytosis by the intravascular macrophages of the liver and spleen when they finally begin to penetrate into the blood stream. That the delayed death is the result of this action of the antibody and is not dependent upon any hypothetical tissue immunity will become evident in a moment when we described experiments on passive immunity in leucopenic animals.

We have said that the animals of the group just described were tested and survived local infection with much larger doses of pneumococci before benzol-treatment was begun, and their serum at that time was active in passively protecting non-immune controls against infection. Since these same animals lost their ability to prevent the growth of the bacteria and to overcome the infection after having been treated with benzol, it became a matter of first importance to determine whether their serum had lost its protective power. This was a crucial point, for if the benzol treatment had destroyed not only the leucocytes but also the power of the serum to protect non-immune animals against infection, the fact that the treated animals succumbed to the infection could not have been regarded as proof that they did so purely because the participation of the leucocytes had been prevented. We have said that the agglutination titre of the immunized animals was not appreciably altered by benzol treatment, but it was nevertheless necessary to examine more directly the protective power of the serum, for we are dealing here with survival immunity which depends upon destruction of bacteria, and it is not generally agreed that agglutination is a necessary part of this process, or that agglutinin is identical with the antibody which promotes bacterial destruction.

We therefore studied the reaction of *animals passively immunized with serum from the immunized benzol-treated group* (group 4 above). On the day on which the test infection was to be given to the benzol-treated animals they were bled from incisions in the vessels of the ear, and 4 to 8 c.c. of the serum of each animal was injected intravenously into a corresponding non-immune control. After the lapse

of an hour these passively immunized rabbits were infected intradermally with the same dose of the same culture at the same time as the benzol-treated animals from which the serum had been obtained. We need not dwell upon the results of this control series other than to say that this small amount of serum from sufficiently immunized, benzol-treated animals served to protect effectively non-immune controls possessing leucocytes. Their lesions remained localized, their blood remained sterile and they survived the infection, while the benzol-treated animals from which they had received the serum succumbed in spite of the fact that their bodies contained far more of the antibody and in a more concentrated form; for it is obvious that the small amount of immune serum with which the normal controls were passively immunized was greatly diluted by the volume of their own plasma, lymph and tissue fluids. It may be mentioned in addition that the local lesion in the passively immunized animals was always much smaller than that in the actively immunized animals untreated with benzol. Indeed, in many cases it was almost impossible to detect any macroscopic lesion at all (fig. 3), although microscopic inflammation and active phagocytosis were always present. The relative minuteness of the local reaction in animals passively immunized to the pneumococcus, in contrast to the pronounced inflammatory reaction in actively immunized animals, has been described by one of us in another connection (8), and the significance of the observation as evidence that the exaggerated inflammation of allergy is not necessary for the protection of the body was there pointed out. In the present experiments we have repeatedly had the opportunity to confirm that observation.

In addition to the type of experiment just described, we demonstrated the potency of the protective power of the immunized, benzol-treated animals' serum by another type of passive immunization which, as we have said, really consisted in *infecting non-immune animals with pneumococci sensitized by the serum of the immunized, benzol-treated animals* (for details of technique see description of procedure in group 5 above). No discussion of this series is necessary other than to say that in these animals the infection was sharply localized, septicaemia did not occur, the bacteria were rapidly ingested and destroyed by the phagocytes which assembled at the site, and the animals survived,

whereas the leucopenic animals from which the serum was obtained succumbed to the same infecting dose of bacteria. Controls infected with the same dose of pneumococci suspended in normal rabbit serum or in serum from non-immune, benzol-treated animals invariably died with an infection which ran a course differing in no way from that in animals in which the infecting pneumococci were suspended in broth. The lesions in the animals infected with the "sensitized" pneumococci resembled those in the animals passively immunized intravenously in being much less conspicuous than the lesions in actively immunized animals (fig. 2). From these experiments it was impressively clear that one single drop of serum from the immunized, benzol-treated animals when injected simultaneously with bacteria was sufficient to protect a non-immune animal possessing leucocytes, whereas the entire volume of the plasma and tissue fluids of the animal from which the serum was obtained was powerless to prevent a fatal outcome in that animal in the absence of the leucocytes. That exposure to immune serum does not destroy the viability of pneumococci is well known, and it will be seen below that when animals without leucocytes are similarly infected with "sensitized" bacteria, or when they are passively immunized intravenously with potent immune serum, the infection invariably proceeds to septicaemia and death.

Finally a word about *the course of infection in non-immune, leucopenic* animals. Normal rabbits were treated with benzol along with the immunized group, and when their leucocytes had been reduced to the level necessary for the production of leucocyte-free lesions they were infected intradermally with the same dose of pneumococci as that given to the groups previously described. In these non-immune, leucopenic animals the lesions resembled those in the immunized, leucopenic group in that the inflammatory oedema and hyperaemia were less marked than in animals untreated with benzol; but the lesions spread progressively over the sides of the animals, often became purpuric, and septicaemia and death occurred somewhat earlier than in most of the normal controls. Microscopically, the lesions showed that, in contrast to the manner in which the bacteria adhered to each other and remained for hours localized at the site of infection in the immunized, leucopenic animals, in the non-immune, leucopenic animals they separated from each other at once after division and

drifted rapidly through the tissues to a great distance from the site at which they were injected (fig. 5). This shows that there is a flow of tissue fluid in the infected skin of benzol-treated animals, in spite of the fact that inflammatory oedema is ordinarily less pronounced than in untreated animals, and demonstrates in addition that the prolonged immobilization of the bacteria in the immunized, leucopenic animals was due to the operation of the mechanism of local fixation described above rather than to any effect of benzol-treatment. Even when no

TABLE I

Course of infection following intracutaneous injection of 0.1 cc. of a 1–100 dilution of culture of virulent Type I pneumococcus

TIME AFTER INJECTION OF BACTERIA	I NON-IMMUNE CONTROL	II BENZOLIZED NON-IMMUNE	III BENZOLIZED ACTIVE IMMUNE	IV NORMAL RABBIT GIVEN 7 CC. SERUM FROM III INTRAVENOUSLY	V NORMAL RABBIT INFECTED WITH BACTERIA SENSITIZED BY SERUM OF III	VI NON-BENZOLIZED ACTIVE IMMUNE
hours						
7	+	+	0	0	0	0
18	+	Dead	0	0	0	0
31	Dead		0	0	0	0
48			0	0	0	0
62			+	0	0	0
			Moribund			
				Survived	Survived	Survived

"+" indicates positive blood culture.

"0" indicates negative blood culture.

The blood of the immunized leucopenic rabbit (III) remained sterile for over two days after the non-immune controls (I and II) had developed septicaemia, but the bacteria immobilized in the leucocyte-free lesion of III continued to proliferate uninterruptedly and the animal finally developed septicaemia and died, although its serum was potent in protecting non-immune animals possessing leucocytes (IV and V).

inflammatory oedema could be detected microscopically the spread of the bacteria was progressive from the beginning. However, when oedema was present the spread was more rapid, the bacteria being carried by gravity downward through the tissues with the oedema fluid, precisely as described by Rhoads and Goodner (31) in pneumococcal infection in non-immune animals possessing leucocytes. We shall return to this method of spread of bacteria in the discussion of the present paper.

That the infection ordinarily progresses slightly more rapidly in leucopenic, non-immune animals than in non-immunes possessing leucocytes is a matter of some interest, for it is well known that there is practically no phagocytosis of virulent Type I pneumococci in the absence of the immune antibody. The bactericidal properties of leucocytic extracts, however, have been established by numerous investigators (28) (29), and we ourselves, in another connection, have carried out experiments on the action of leucocytic extracts on a variety of different bacteria, which will be reported in another place. It may be stated here that we found that the extracts exert a very definite bactericidal and growth-inhibiting effect upon the Type I pneumococcus. It is not extremely potent, but it is none the less distinctly demonstrable, and it seems reasonable to believe that the liberation of this substance from the myriads of disintegrating leucocytes at the site of infection in the non-immune controls may possibly be responsible in some measure for the slightly slower progress of the infection in that group as compared with non-immune animals with leucocyte-free lesions.

THE COURSE OF INFECTION IN PASSIVELY IMMUNIZED LEUCOPENIC ANIMALS AND IN THEIR CONTROLS

The experiments which have been described make it clear that, in the absence of the leucocytes, actively immunized animals are unable to prevent the continued growth of pneumococci deposited in their tissues, and they therefore succumb to the infection. However, even in the absence of the leucocytes the immunized body retains to a marked degree its power to inhibit the prompt and free spread of the bacteria, a power which the non-immune body does not possess. The studies which one of us has reported in another place (8) have shown that the primary agent responsible for the inhibition of spread of pneumococci in the immune body is the immune antibody. Since those studies were carried out on animals possessing leucocytes, we felt it to be of importance to determine whether the immune antibody would be as potent in restraining the dissemination of bacteria in the absence of any participation of the leucocytes, or of any theoretically possible alteration of the tissues themselves which active immunization might bring about. We therefore carried out experiments in which

normal, non-immune rabbits were deprived of their leucocytes by means of benzol and were then infected intradermally, after having been passively immunized with antipneumococcal serum. Two types of experiment were performed. In the first group the intravenous method of passive immunization was employed. When the circulating leucocytes had been reduced by benzol to the level necessary to prevent emigration at the site of infection, the rabbits were given intravenously 15 to 20 c.c. of either potent, commercial Type I antipneumococcal horse serum, or fresh serum from rabbits intensively immunized by the method outlined above. An hour or two after having received the serum they were infected intradermally with 0.1 c.c. of a 1:10 or 1:1000 broth dilution of a culture of virulent Type I pneumococci. In the second group the non-immune leucopenic animals received no serum intravenously, but were infected intradermally with 0.1 c.c. of a culture of pneumococci diluted in the final step in immune horse or rabbit serum instead of in broth, as described above. As controls for each of these groups, normal rabbits possessing leucocytes were given the same dose of the same serum and culture in the same manner and, in addition, other normal rabbits were infected with the same dose of pneumococci in the absence of any treatment with immune serum.

The blood cultures of the *normal controls which received no immune serum* became positive after a few hours; the lesions were the large, spreading, and often purpuric ones characteristic of the dermal infection in non-immune animals, and all of the animals died within 24 to 36 hours after infection.

The lesions in the *normal controls which received immune serum* either intravenously or only intracutaneously along with the pneumococci were inconspicuous; they never became purpuric and never spread progressively, the blood remained sterile and the animals always survived.

In the *leucopenic animals which received immune serum* either intravenously or only intracutaneously the lesions were still less conspicuous during the first 24 hours than those of the preceding group, but they spread gradually thereafter and often became purpuric just as in the case of the actively immunized leucopenic animals described above. The blood culture was always sterile during the first 24 hours but

was ordinarily positive at 48 hours, and the animals invariably died with heavy septicaemia and with leucocyte-free lesions teeming with pneumococci. In one instance, in which a second intravenous injection of immune serum was given on the day following the first, the blood remained sterile during four days, but the bacteria continued to proliferate at the site of infection and the lesion became progressively larger and more purpuric. The average period of survival of these passively immunized leucopenic animals was from two to three days after infection. Microscopically, colony-like growth of the bacteria and their inhibited movement through the tissues were observed precisely as in the actively immunized leucopenic animals described above.

From these experiments it is clear that the presence of immune serum serves to inhibit markedly the spread of pneumococci in previously normal tissues, and this in the complete absence of the leucocytes at the site of infection and in the face of much less inflammatory reaction than occurs even in the non-immune body in which the bacteria spread rapidly. However, in the absence of the leucocytes, highly potent immune serum fails to prevent the steady growth of the bacteria in the tissues. These experiments, further, make it clear that the longer survival of the actively immunized leucopenic animals, as contrasted with non-immune ones, was not the result of some obscure and hypothetical "tissue immunity" developed during active immunization, for the passively immunized leucopenic animals reacted quite like the actively immunized ones, and in the former the tissues had never been subjected to active immunization and differed from non-immune tissues only in having been brought into contact with immune antibody.

DISCUSSION

The present experiments demonstrate that in the absence of the leucocytes the growth of virulent Type I pneumococci proceeds progressively in the tissues of the highly immunized body even in the presence of all of the other elements of inflammatory exudate, and the animal dies of the infection even though its plasma is potent in passively protecting non-immune animals possessing leucocytes. In Type I pneumococcal infection, therefore, the leucocytes are essential

for the operation of the prime protective function of the state of immunity, *i.e.*, the destruction of those bacteria which find their way into the tissues. Acquired immunity creates no condition of the fluids or the fixed tissues which can prevent the progressive and overwhelming growth of the bacteria in the absence of the leucocytes. As for that important accessory function of the immune state, the prevention of the prompt and free spread of the bacteria from the site at which they lodge, the experiments show that that is accomplished *primarily* through the agency of the immune antibody, and this process is, therefore, clearly manifest in the complete absence of leucocytes at the site of infection, and even in the absence of microscopically detectable inflammatory oedema or fibrin. However, since the antibody is not itself bactericidal, but acts only to hold the bacteria at the site until the leucocytes can emigrate to attack and destroy them, in the absence of the leucocytes growth of the immobilized bacteria proceeds uninterruptedly until, eventually having formed great colonies and masses in the tissues, they penetrate into the blood stream and the animal dies with septicaemia. The experiments, especially those in the passively immunized, leucopenic animals, show in a striking way the remarkable efficacy of the antibody in preventing the spread of the bacteria, but they also make it clear that even in the highly immune body, whether the immunization be active or passive, the immobilizing effect of the antibody must be supported by the destruction of the bacteria by the leucocytes or else septicaemia and death are inevitable. The sterilizing activity of the leucocytes (which, of course, is made possible by the opsonizing antibody) constitutes the only *necessary* condition for survival immunity in this infection. The bacteria may spread for a time through the tissues and invade the blood stream, as they do in incompletely immunized animals, but if their free proliferation is eventually suppressed by the leucocytes, the animal will survive. Nevertheless, it is obvious that the successful immobilization of the bacteria at their point of entry into the tissues is a very important part of immunity, for their spread not only causes more extensive local destruction of tissue (which, in vital spots, can lead in itself to death) but, in addition, every septicaemia means an enhanced opportunity for metastatic infection of distant organs.

Inflammation, of course, serves the exceedingly important function

of bringing to the site of infection the phagocytes which, in the presence of the immune antibody (opsonin) ingest and destroy the bacteria. The antibody itself may also escape from the blood together with the other elements of the inflammatory exudate, but it is unlikely that any excess of antibody is necessary for protection above that which, in the immune body, permeates the fluids of the uninflamed tissues everywhere, as shown by Freund (15), Cook and Smith (16) and Douglas and Simson (17). That the tissue fluids move freely in the benzolized, leucopenic animals is clear from the fact that in such animals in the absence of the immune antibody the bacteria drift progressively through the tissue spaces of the skin to a distance of 10 cm. from the site of infection and enter the blood stream as promptly as they do in normal controls. Certainly, in our experiments agglutination of the bacteria in the tissues of the immune, leucopenic animals was brilliant even when inflammatory oedema, if present at all, was too slight in amount to be detected microscopically. It is probably impossible, however, to inject the 0.1 c.c. of fluid containing the bacteria without causing some slight inflammatory oedema.

Although the bacteria grow progressively in the immune tissues in the absence of the leucocytes, it is nevertheless conceivable that the phenomenon of agglutination interferes to some extent with their free proliferation, not in an actively bactericidal way, but by interfering with their nutrition. *In vitro* studies have, up to the present, been unsuccessful in demonstrating satisfactorily any specific growth-inhibiting effect of immune serum upon bacteria, but the available experimental methods are themselves not as satisfactory as one might desire. Barber found no retardation in the growth rate of pneumococci by immune serum during the first several generations, but his experiments (22) throw no clear light upon the rate of growth of the bacteria in immune serum after large clumps have been formed. It seems not unlikely that when bacteria grow adhering to each other in closely packed masses, as they do in the tissues of the immune body containing agglutinin, the nutrition of those in the center of the mass may be interfered with and their growth correspondingly inhibited, while the prompt separation of the daughter cells which occurs when pneumococci divide in the non-immune body (8) may enhance the opportunity of each new cell to obtain nutriment from the surround-

ing tissue fluids as it drifts through them. The experiments show plainly, however, that any inhibition of growth which may result from agglutination is not at all sufficient to protect the body against infection in the absence of the leucocytes.

As for the rôle of inflammation in *mechanically* preventing the spread of the bacteria, it is clear from these experiments, as well as from those of a different nature previously reported by one of us (8), that the immediate fixation of the bacteria is entirely independent of such mechanical barriers as a wall of leucocytes, a fibrin network in the tissues, or thrombosed lymphatics, for in the immune, leucopenic animals the bacteria remained where they were placed for hours in the absence of all of these factors. On the contrary, in the non-immune animal possessing leucocytes, they spread rapidly and progressively from the site in spite of the most intense inflammation and the attendant presence of all three factors. In a recent paper concerned with the effects of a previously prepared area of inflammation in retarding the spread of a dye injected into it, Menkin, discussing the abstract of our report of the present experiments before the Society for Experimental Pathology, states that our conclusions regarding the immobilization of bacteria "seem to be based on the assumption that the presence or absence of leucocytes is the sole criterion of inflammation" (18). Again, in the same paper, Menkin states that "several years ago Rich and McCordock, expressed strong doubts as to the proof of the ability of the inflammatory reaction to limit the dissemination of bacteria. Rich (8), probably in view of the amount of evidence recently brought forth, has since admitted the fact." Both of these statements must have arisen from hasty reading of the quoted papers, for in our abstract referred to by Menkin it is stated specifically that we found that the immobilization of bacteria in the immune body occurs in the "absence of local inflammatory exudate *of any sort*" (26) and in the paper of Rich and McCordock which Menkin quotes there is the following plain statement in the paragraph discussing the mechanical effects of inflammation in inhibiting the spread of bacteria; "Undoubtedly, once walled-off their further spread is hindered, but this does not touch the question of why they remain localized long enough to become walled-off" (23). We have repeatedly stated specifically that *once inflammation is well established* it acts to inhibit the free

movement of bacteria (7), (8), (23). Indeed, we ourselves several years ago reported experiments which showed clearly the effect of inflammation in inhibiting bacterial invasion (7). This fact that an area of *previously prepared* inflammation can inhibit the growth and invasion of bacteria injected into it is an old observation. It was described forty years ago by Issaeff (5) and has been repeatedly confirmed since (1), (2), (4), (7), (19), (27). That fact has never been called into question by us. What we have questioned is the statement that inflammation appears quickly enough about invasive bacteria which lodge in *previously uninflamed* tissues (as is the situation in natural infection) to prevent the rapid spread of those bacteria which, in the non-immune body has been shown to occur within a matter of minutes; and we have not altered our opinion that it does not. On the contrary, our own experiments on this point (7), as well as the early ones of Cobbett and Melsome (3) and those of all subsequent investigators including Menkin himself (6), show consistently that when bacteria or other foreign substances are injected into the non-immune body *together with* an inflammatory irritant which will stimulate the rapid development of inflammation, the spread of the bacteria or foreign material is not retarded even by that accelerated inflammation. In many cases, indeed, the spread is hastened. It is only when the inflammatory irritant has been allowed to act upon the tissues for a period of time *before* the bacteria are injected into the area that their spread is inhibited. From this it is clear that there must exist in the immune body some other mechanism which acts to hold the bacteria immobilized until the accessory mechanical barriers of inflammation have time to develop to an effective degree, and this especially in view of the fact that the present experiments, as well as others which we have previously reported (8), (13), show that in the immune body immobilization of the bacteria occurs in the absence of fibrin deposition or of lymphatic thrombosis, and even when the amount of inflammation at any point of time after infection is *far less* than that in the non-immune body in which the bacteria spread rapidly and progressively. During the pre-inflammatory period after bacteria lodge in the tissues, *i.e.*, before inflammation has been developed sufficiently to assist mechanically in inhibiting their spread, the bacteria are held at the site in the immune body, and they move

freely from it in the non-immune one; and it is the mechanism in operation during this period which has occupied our attention particularly, and upon which the present experiments bear. The precise nature of this immobilizing mechanism has been discussed by one of us in another place (8). There is no conflict between these experiments and the experiments of others on the effect of established inflammation in limiting mechanically the spread of bacteria. The latter experiments have been carried out on non-immune animals, while we have been interested in studying a phenomenon which is exhibited only by the immune body, and which is one of the prime manifestations of the immune state. Intense inflammation occurs about pneumococci in both the immune and the non-immune body and, therefore, cannot be regarded as primarily responsible for that remarkable local immobilization of the bacteria which occurs only in the immune body, and even under conditions in which there is far less inflammation than in the non-immune one (8) (13). When inflammation is finally well established in the immune body it undoubtedly assists in preventing the spread of many types of bacteria, as we have said, and the studies of Menkin have contributed to our understanding of the mechanism through which this is effected. However, it is important to point out that the degree to which even established inflammation is able to inhibit bacterial invasion varies markedly with different types of bacteria; and it is of particular interest in the present connection that Clark found that a site of previously prepared inflammation did not at all inhibit the invasion of virulent Type I pneumococci in the non-immune body. Cobbett and Melsome (3) had previously reported the same fact and Clark, in a more pointed investigation, found that, although the spread of streptococci was markedly inhibited when the bacteria were deposited in the rabbit's pleural cavity which had been previously inflamed by aleuronat, there was no inhibition of spread of pneumococci from such inflamed cavities, not even when an infecting dose as small as 0.0000001 c.c. of culture was deposited in a pleural cavity which had been inflamed for various periods of time up to 72 hours before the bacteria were introduced. "The prepared cavities were not resistant to injections of pneumococcus" writes Clark, "neither those containing an exudate predominantly polymorphonuclear with an inflamed pleura nor those containing an exudate with

many mononuclear cells and a thickened pleura containing granulation tissue and many macrophages. . . . . There was rapid invasion of the blood stream from both prepared and normal cavities; this accumulation of cells did not localize the infection" (30). These experiments serve to reinforce the fact that inflammation cannot be regarded as the primary localizing mechanism in the body immune to virulent Type I pneumococci.

Indeed, it seems clear that at least one element of the inflammatory exudate—oedema—tends definitely to further the spread of the bacteria. When the masses of bacteria in the lesions of the immunized leucopenic animals have become very large with the passing of time, separation of the growing bacteria from each other as they divide at the periphery of the colonies becomes more prominent than in the earlier hours of the infection, and the tendency to drift in the tissue spaces as single diplococci, or as small clumps, is more evident; and at this time oedema, also, is more evident. It might be thought that the occurrence of inflammatory oedema would assist in maintaining the coherence of the bacteria to each other by bringing more antibody to the site, but the more marked the oedema the more prominent is the drifting of the bacteria, which float in the oedema fluid that separates widely the cells and fibres of the tissues, and are carried with the fluid as it moves through the tissue spaces. Rhoades and Goodner (31) have emphasized the rôle which the fluid of inflammatory oedema plays in transporting pneumococci from the site of infection in the non-immune body under the influence of gravity, and our own observations are entirely in accord with theirs. Although, in the immune body, the antibody acts to inhibit the free movement of the bacteria, here, too, oedema tends to promote their spread through the tissues; but even in the face of oedema the lesion in the immune animal possessing leucocytes never attains the size of that in the non-immune animal, for, in the former, immune antibody acts not only to oppose the separation and free movement of the bacteria but it also opsonizes them, and the progress of the lesion is soon limited by the destruction of the bacteria by the leucocytes. In the immunized leucopenic animal, however, there are no leucocytes to restrain the proliferation of the bacteria, and in the animals of this group in which inflammatory oedema was conspicuous the local spread of the bacteria

was always greater than in those in which oedema was minimal in amount, and in the former the floating of the bacteria in the oedema fluid was plainly visible (fig. 15). Likewise, in the non-immune leucopenic animals in which oedema was more prominent, the bacteria spread more rapidly through the tissues locally than in the animals of this group in which oedema was very slight or absent. However, even in the latter, penetration of the bacteria into the blood stream was prompt, and septicaemia always developed several hours after infection. In contrast, in the immune leucopenic animals septicaemia was always inhibited for 36 hours or more, regardless of whether or not oedema was prominent. Oedema, therefore, though it distinctly furthered the local spread of the bacteria through the tissues, did not appear to exert any influence upon their power of penetrating the vascular endothelium.

We have shown elsewhere (7) that in animals possessing leucocytes not even the accelerated and exaggerated inflammation of allergy is developed rapidly enough about bacteria to prevent their growth and spread in the absence of the immune antibody and, on the other hand, that both their growth and spread are prevented in the presence of the immune antibody even when inflammation is far less in amount than in the non-immune body in which growth and spread proceed uninterruptedly (8) (13). The present experiments on immunized leucopenic animals add further very clear evidence that the exaggerated inflammation of allergy is not necessary for immunity, for even in the instances in which only a relatively small number of leucocytes appeared at the site of infecton, and in which inflammation was trifling in amount as compared with that in the non-immune controls which succumbed, or that in the immune controls possessing leucocytes which survived, the bacteria were destroyed and the lesions were sterilized. Microscopical study showed plainly that when sterilization of the lesions occurred it was invariably directly dependent upon the escape of leucocytes at the site.

Since the ability to sterilize the lesion bore no direct relation to the amount of benzol the animal had received (animals in which emigration of leucocytes occurred would sterilize their lesions even though they had received twice the amount of the drug given to others which succumbed because of the absence of leucocytic emigration) there

is no reason to believe that the free proliferation of the bacteria in the immunized leucopenic animals was a result of the breaking down of any hypothetical anti-bacterial "tissue immunity" by benzol. Furthermore, the sections offer no evidence that the tissues at the site of infection in the actively immunized animals possessing leucocytes are in any degree more resistant to damage by the pneumococci present in the lesions than are the tissues of the immunized benzol-treated animals. On the contrary, at any stage of the infection in which the numbers of bacteria in the sections are approximately the same, the tissues at the site of infection in the immune and non-immune animals with leucocytes are much more severely damaged than are those of the corresponding benzol-treated animals. Indeed, during the early hours of the infection the local damage is more marked in the immunized animals possessing leucocytes than even in the non-immunized benzol-treated animals in which many more bacteria are present at any given time. The damage in the former, however, except in the case of allergic animals, is produced mainly by the mechanical disruption of the tissues by the abundant inflammatory exudate. This local damage is much less pronounced in passively immunized animals or in desensitized animals than it is in allergic animals (8) (13), but even in the former, in which allergy is excluded from the picture, the local damage is greater than that which occurs about the pneumococci in even the non-immune benzol-treated animal in which the inflammatory exudation is suppressed. A comparison of the local lesions in actively immunized and non-immune animals with leucocytes and without leucocytes fails to reveal the slightest evidence that the tissues at the site of infection in immunized animals with leucocytes are in any degree more refractory to the effects of a given number of bacteria than are the tissues of non-immune animals with leucocytes, or those of the benzol-treated animals. There is, therefore, in these experiments no evidence whatever that benzol destroys any hypothetical local tissue immunity to the bacterial products. It is true that Zinsser (33) has shown that when the pneumococcus undergoes lysis *in vitro* a toxic substance is liberated which, when injected into the skin, produces local tissue damage, and we have repeatedly had occasion to confirm this observation. It may be stated, however, that in order to obtain the necrotizing substance in readily demonstrable

concentration enormous numbers of pneumococci must be lysed in very small amounts of fluid. Thus Parker (34) recommends that the sedimented pneumococci obtained by centrifugalizing heavy broth cultures be allowed to undergo lysis in an amount of fluid equal only to the volume of the packed bacterial cells. 0.1 c.c. of the resulting lysate will cause a small area of necrosis when injected intradermally. The production of local necrosis by the injection of 0.1 c.c. of the lysate represents, therefore, the result of the injection of the dissolved bacteria contained in at least 5 c.c. of a heavy, undiluted culture into an area about 1 cm. in diameter, obviously a concentration of bacterial lytic products which can scarcely occur in such a small area at any site of infection. As a matter of fact, it is quite clear that, although this concentrated lysate causes necrosis of the skin, very considerable numbers of pneumococci fail to do so in any appreciable degree in the absence of hypersensitivity either in the rabbit or in man. In human pneumococcal pneumonia, for example, in spite of the presence of large numbers of pneumococci, necrosis of the alveolar walls rarely, if ever, occurs in the absence of vascular obstruction, whether in non-immune infants or in adults. But regardless of this and of any effects which the toxic substance may exert upon more susceptible tissues at a distance from the site of infection (32), Parker has shown that the toxic principle may be neutralized by antitoxin, and any protection against its effects which the immune body may possess is explainable, therefore, as a result of the action of humoral antibody without invoking any hypothetical state of acquired tissue immunity.

We have pointed out the rôle which the immobilizing effect of the immune antibody plays in prolonging the period of survival of immune, leucopenic animals as compared with non-immune animals, and we have demonstrated histologically that the bacteria are actually immobilized. However, it must be borne in mind that this local immobilization is undoubtedly greatly reinforced by the "increased clearing capacity" of the blood of the immune body. It is well known that bacteria injected into the blood stream of the immune body are removed from the circulation with much greater rapidity than in the non-immune body. Bull (24) has emphasized the rôle that intravascular agglutination may play in segregating bacteria which enter the circulation of the immune body, and Cannon, Sullivan and

Neckermann (20), in a recent careful study of the matter, have drawn attention to the importance of the macrophages of the liver and spleen in removing bacteria from the blood stream of the immune body. In our benzol-treated immunized animals both of these phenomena occurred. We have found agglutinated pneumococci in the blood of immunized, leucopenic animals during the period of septicaemia; and, in common with Selling, we have observed that the macrophages of the liver and spleen are very resistant to benzol, and we have found that in the immunized benzol-treated body they avidly ingest and destroy pneumococci which enter the blood stream (figs. 16, 17). It is therefore clear that when small numbers of bacteria finally begin to penetrate into the blood from the lesions in the leucopenic animals, they encounter an accessory protective mechanism which acts to delay the death of the animal.

What, finally, of the conclusion of Winternitz and Kline that the white blood cell is not necessary for immunity to the pneumococcus, as stated in the introduction of this paper? The observation of these investigators that their actively immunized, benzol-treated rabbits survived the test infection is clear cut and precise, but there are several circumstances which, in the light of what we have learned, permit us perhaps to understand the discrepancy between their conclusion and our own. In the first place, the pneumococcus used in their experiments must have been of very low virulence, for they immunized their rabbits by injecting living organisms, beginning with doses of 0.5 c.c. of undiluted culture. Rabbits, however, are so susceptible to really virulent Type I pneumococci that they regularly succumb to infection with 0.1 c.c. of culture diluted 1–1,000,000. Winternitz and Kline do not state the degree of virulence of their culture, nor do they mention the type of the pneumococcus. However, it may be stated here that in studies which we have been carrying out on Type II and Type III pneumococcal infection in the rabbit we have found that immunity to these types also is destroyed when the leucocytes are abolished.

In the second place, all of the test inoculations in their experiments were made intravenously, which obviously delivered the bacteria directly not only to any leucocytes which remained in the circulation (and the circulating leucocytes in their protocol ranged from 280 to

580 per cubic millimetre following infection) but also to the phagocytic intravascular macrophages of the liver and spleen which, as we have shown above, continue to exercise actively their phagocytic function in the benzolized animal. In their experiments, therefore, pneumococci of low virulence were delivered directly to those phagocytic polymorphonuclear and macrophage cells which remained active in the blood stream, and the survival of their immunized animals is readily explainable on the basis of phagocytosis, without having recourse to the assumption of the existence of any hypothetical extracellular bactericidal agency such as that suggested by Winternitz and Kline.

SUMMARY

When the circulating leucocytes are sufficiently reduced by benzol none emigrate at a site of infection, and the character of the protection afforded by the immune state, independently of that conferred by the activity of the leucocytes, can be studied. Immunized animals were therefore treated with benzol to remove their leucocytes, and were then infected intradermally with virulent Type I pneumococci. It was found that in these immunized, leucopenic animals the presence of immune antibody influences profoundly both the character of bacterial growth and movement, and also the course of the infection. The bacteria, as they proliferate, adhere to themselves and apparently to the tissues as well, and are thus held fixed at the site of inoculation for hours after non-immune controls have died with septicaemia. The immediate local immobilization of the bacteria is demonstrable even in the absence of microscopically detectable inflammatory exudate, or thrombosis of lymphatics. These latter factors are, therefore, not primarily responsible for the immobilization of the bacteria in the immune body, though when they are finally established they undoubtedly serve to assist in inhibiting the spread of the bacteria.

Since the phenomenon of immobilization occurs in passively as well as in actively immunized animals, it is the antibody content of the fluids of the immune body which is primarily responsible for this prevention of the prompt spread of the bacteria. However, in the absence of the leucocytes the growth of the immobilized bacteria proceeds uninterruptedly until, great colonies and masses of pneumo-

cocci having been formed at the site of infection, the bacteria eventually penetrate into the blood and lymph streams, and *the animal dies with septicaemia even though its plasma is potent in passively protecting non-immune animals possessing leucocytes.* If only a relatively few leucocytes appear at the site of infection (far fewer than the number which appear in the lesions of non-immune controls which succumb to the infection) the bacteria, which are opsonized by the antibody, are rapidly ingested and destroyed, and the lesion is sterilized.

In addition to its opsonizing power, the humoral antibody, therefore, performs the important protective function of preventing the immediate spread of the bacteria throughout the body, holding them fixed at the site where they lodge until the phagocytic leucocytes are able to reach the spot and destroy them. It is, furthermore, the humoral antibody which, with the coöperation of the intravascular phagocytic macrophages of the liver and spleen, brings about the rapid segregation and destruction of bacteria which do penetrate into the blood stream, thus further inhibiting the development of septicaemia and metastatic infection; but acquired immunity creates no condition of the fluids or fixed tissues which can prevent the progressive and overwhelming growth of the bacteria in the absence of the phagocytes.

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EXPLANATION OF ILLUSTRATIONS

(Photographs by Mr. Milton Kougl)

FIG. 1. Actively immunized leucopenic animal 48 hours after injection of 0.1 c.c. of 1:100 dilution of culture of Type 1 pneumococcus intradermally. The lesion spread much more slowly than that in the normal control, but eventually extended over an area 10 cm. in diameter and was very haemorrhagic. At the time of the photograph and for 7 hours afterwards, the blood was sterile but the animal then developed a heavy septicaemia and died. The blood culture of the control showed 460 colonies per cubic centimeter at 7 hours, and the animal was dead at 31 hours.

FIG. 2. Normal animal which received same dilution of same culture at same time as rabbit of fig. 1, the final dilution being made in the serum of rabbit of fig. 1. No macroscopic lesion was visible at any time; the blood remained sterile, and the animal survived. Ink spot marks site of infection at end of 48 hours.

FIG. 3. Normal animal passively immunized by intravenous injection of 7 c.c. of serum from rabbit of fig. 1, and then injected with same dose of same dilution of culture at same time as rabbit of fig. 1. Ink spot marks site of infection at end of 48 hours. The lesion was at no time more marked than in the photograph—a slightly oedematous area about 1 cm. in diameter. The blood remained sterile and the animal survived.

FIG. 4. Colony-like growth of Type 1 pneumococci in lesion of actively immunized leucopenic animal 8 hours after injection of 0.1 c.c. of a 1:100 dilution of culture.

FIG. 5. Diffuse growth of Type 1 pneumococci in lesion of non-immune leucopenic animal 8 hours after injection of 0.1 c.c. of a 1:100 dilution of culture.

FIG. 6. Diffuse growth of Type 1 pneumococci in lesion of normal, non-immune control 8 hours after injection of 0.1 c.c. of a 1:100 dilution of culture. There are abundant leucocytes but no phagocytosis.

FIG. 7. Colony-like clump of Type 1 pneumococci in lesion of actively immunized animal possessing leucocytes, 8 hours after injection of 0.1 c.c. of a 1:100 dilution of culture. Leucocytes cover the clump, and phagocytosis is prominent.

FIG. 8. Marked inflammation with myriads of leucocytes in lesion of non-immune control 19 hours after injection of 0.1 c.c. of a 1:100 dilution of culture of Type 1 pneumococcus. In spite of the inflammation (no phagocytosis, however) the animal developed septicaemia several hours after infection and died 12 hours after the portion of the lesion represented by the photograph was removed.

FIG. 9. Site of infection in non-immune leucopenic animal 19 hours after injection of 0.1 c.c. of a 1:100 dilution of culture of Type 1 pneumococcus (bacterial stain). There is no leucocytic emigration and but slight oedema. The dark band at the bottom of the corium represents diffusely growing pneumococci. See fig. 10. The blood culture became positive 12 hours before the section was taken.

FIG. 10. Shows the diffuse growth of the bacteria in the dark band of fig. 9 (oil immersion).

FIG. 11. Site of infection in immunized leucopenic animal 19 hours after injection of 0.1 c.c. of a 1:100 dilution of culture of Type 1 pneumococcus (bacterial stain). The black spots in the center of the corium are colony-like masses of pneumococci. There were numerous masses of this nature localized at the site of infection. There is no emigration of leucocytes and practically no detectable oedema (most of the spaces in the section are artefacts). There is no fibrin in the tissues and no thrombosis of lymphatics, but the blood culture was sterile when the section was taken, though that of non-immune controls infected at the same time had become positive 12 hours before.

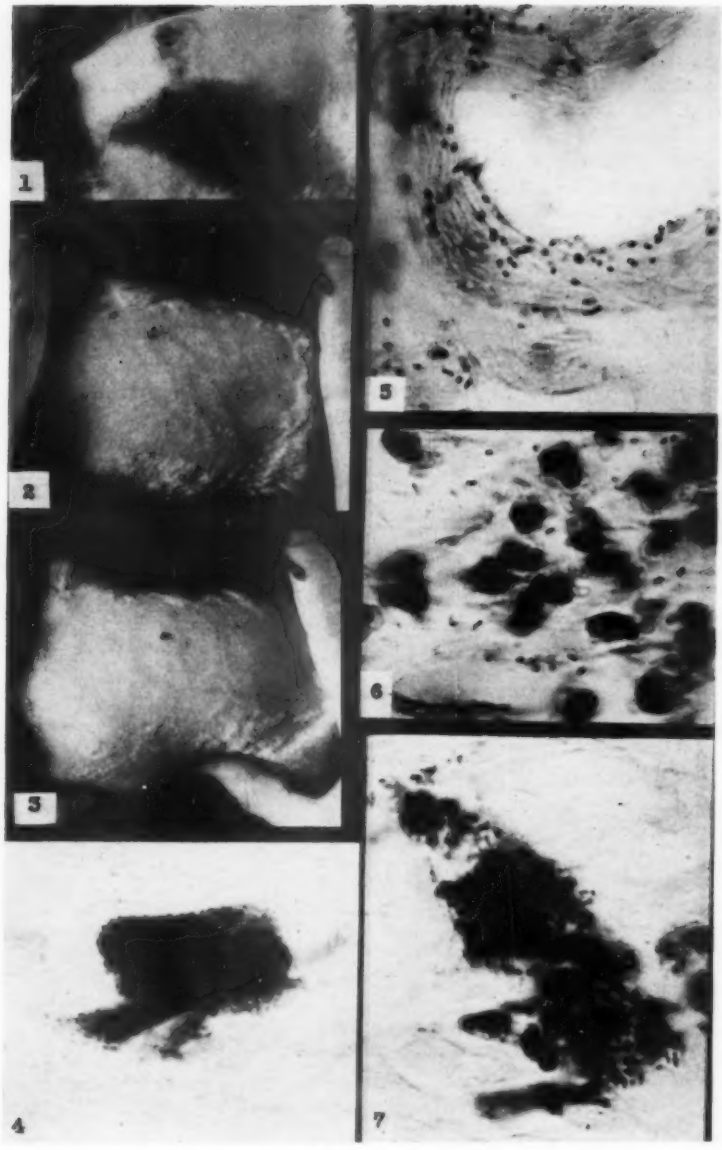
FIG. 12. Shows the colony-like masses of bacteria in fig. 11 (oil immersion).

FIG. 13. Site of infection in immunized leucopenic animal 48 hours after injection of 0.1 c.c. of a 1:100 dilution of culture of Type 1 pneumococcus. The black areas are dense masses of pneumococci filling up the tissue spaces. There is no leucocytic emigration. In other places in the section oedema was more pronounced. The blood culture of this animal remained sterile for over 36 hours, but was positive at the time this section was taken. See figs. 14 and 15.

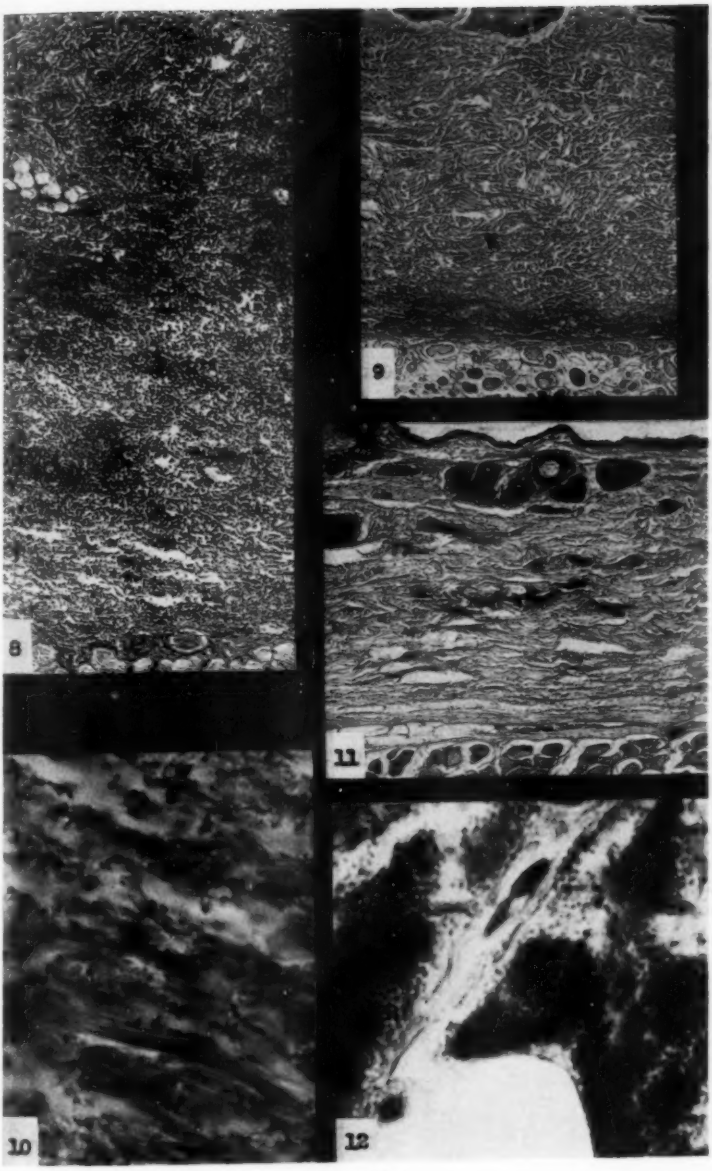
FIG. 14. Sharply outlined, colony-like masses of pneumococci in an area of fig. 13 where oedema was very slight (oil immersion).

FIG. 15. Small clumps of bacteria breaking from margins of colony-like mass and floating in the oedema fluid in an area of the section of fig. 13 where oedema was pronounced (oil immersion).

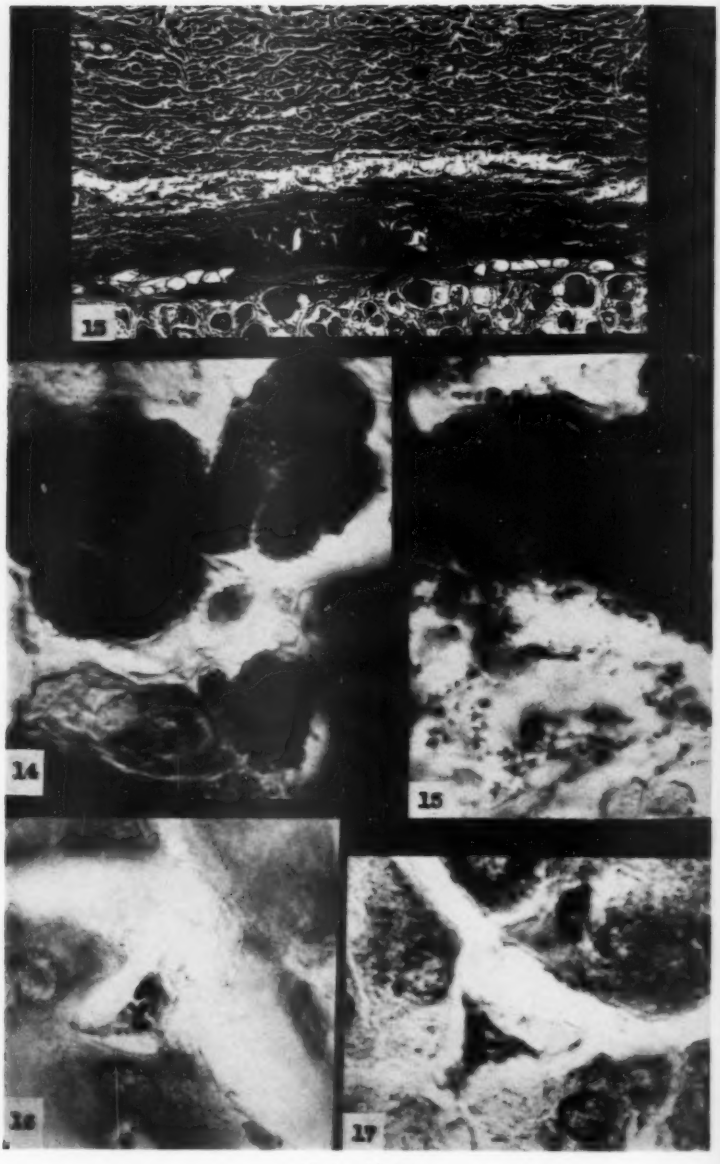
FIGS. 16 AND 17. Phagocytosis of the bacteria by Kupffer cells of the liver in an immunized leucopenic animal 5 minutes after the intravenous injection of Type 1 pneumococci.



FIGS. 1–7



FIGS. 8–12



FIGS. 13-17